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The Journal of Laboratory and Clinical Medicine

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ORIGINAL ARTICLES

SMALLPOX VACCINATION AT THE UNIVERSITY OF CALIFORNIA*

BY J. N. FORCE, M.D., BERKELEY, CALIF.

THE Regents of the University of California require that all intrants shall show satisfactory vaccination scars or other evidence of immunity against smallpox.

In determining what constitutes a satisfactory scar we have not been influenced by the size or character, since complete immunity to smallpox vaccine may exist in an unscarred person or in one whose scar is entirely free from characteristic pits. The duration of immunity, however, varies inversely as the age of the vaccination scar. In a series of 318 persons showing vaccination scars under ten years old, 48 per cent were completely immune as indicated by the cutaneous allergic reaction of immunity, 38 per cent were partially immune as indicated by the secondary vaccinia (vaccinoid), and 14 per cent showed no immunity to revaccination as indicated by the primary vaccinia produced. In a similar series of 444 persons whose scars were between ten and twenty years old, the same percentages were obtained. In a series of 136 persons whose scars were over twenty years old, 38 per cent were completely immune, 27 per cent were partially immune, and 35 per cent showed no immunity to revaccination. Since we have reason to believe that immunity to smallpox is more lasting than immunity to vaccination, we ignore the 14 per cent of nonimmunes and set the limit for revaccination at ten years.

If an intrant has a scar over ten years old he is vaccinated in two spots on the arm, a control spot being scarified but not vaccinated. Observations are made at twenty-four hour and five day periods after vaccination. If either of the vaccinated spots show an areola of 5.0 mm. or over (with or without papule)

*Submitted to the National Research Council by Dr. F. P. Gay, University of California, Member of Committee on Medicine and Hygiene.

at the end of twenty-four hours, which areola (or papule) has decreased at the time of the fifth day observation, it is considered a *reaction of immunity* due to the presence of antibodies against smallpox vaccine in the tissues and the sub-

Fig. 1.—Reaction of immunity twenty-four hours after vaccination. The upper spot is a control. There are 5 mm. areolæ around both vaccinated spots.

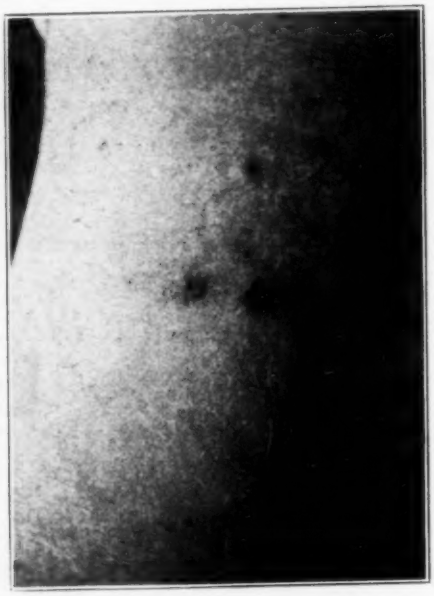

Fig. 2.—Reaction of immunity forty-eight hours after vaccination. The areolæ have increased slightly and there is papule formation.

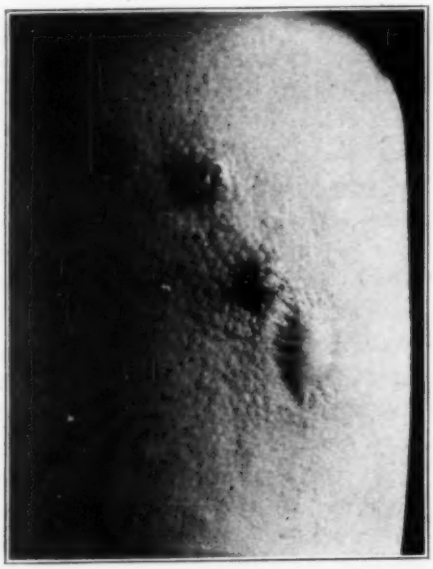

Fig. 3.—Vaccinoid sixth day after vaccination. The reaction has attained its maximum. The previous scar is twenty years old.

ject is excused from further vaccination. If either of the vaccinated spots show an areola at the end of twenty-four hours which develops into a small vesicle which matures on the fifth or sixth day and then rapidly subsides, the reaction is con-

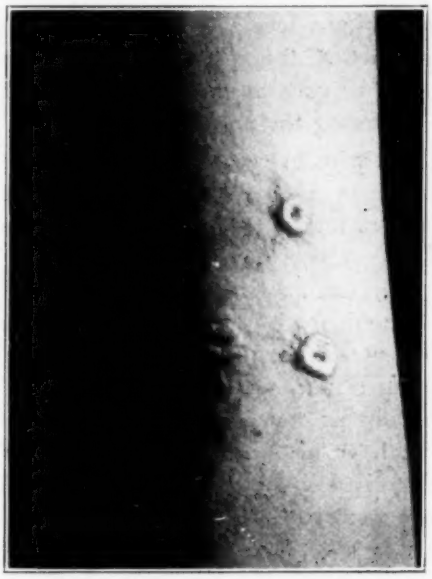

Fig. 4.—Vaccinia sixth day. Compare with Fig. 3.

Fig. 5.—Vaccinia eleventh day. The height of the reaction.

Fig. 6.—Scars resulting from the vaccinia produced by three scarifications 2 mm. in diameter. The scars measure 11 mm. in diameter each.

sidered a *secondary vaccinia (vaccinoid)*. If there is no change until the third day, and then a small areola begins to form, the case will be one of *vaccinia*. If the small areola has not become a vesicle by the fifth day the smallpox vaccine is probably incapable of producing vaccinia, or was not properly brought in contact with the derma. The vaccination is accordingly repeated with fresh vaccine. If the subject is not seen until the fifth day and there are then no signs of a recent local reaction, the vaccination is repeated, for all signs of the reaction of immunity may be gone by the fifth day following vaccination. Inert vaccine is capable of producing the reaction of immunity in an immune subject.

Repeated vaccination with an inert vaccine may finally render the subject immune to potent vaccine, or a small scar may disappear though the immunity persists. In a series of 215 intrants showing no vaccination scar but giving histories of from one to twenty previous vaccinations, one per cent were immune, six per cent were partially immune, and 93 per cent were not immune to smallpox vaccine. In our experience no person who has been given internal vaccination has shown the slightest immunity to smallpox vaccine. In a series of unscarred intrants with a history of smallpox, 36 per cent were completely immune, 40 per cent were partially immune, and 24 per cent showed no immunity to smallpox vaccine. We have never produced a primary vaccinia in a person who showed the characteristic pits of smallpox, but in many instances we have produced the secondary vaccinia (vaccinoid) in such subjects.

In a series of 465 unscarred intrants, claiming never to have been previously vaccinated, *not one person was completely immune*, 1.3 per cent showed a secondary vaccinia indicating partial immunity, and 98.7 per cent showed no evidence of immunity to smallpox vaccination.

This extremely satisfactory record of 100 per cent of vesicle formation in previously unvaccinated intrants, and 99 per cent of vesicle formation in previously vaccinated intrants (unscarred) we consider due to care of the vaccine and uniform vaccination technic.

CARE OF THE VACCINE.

In a series of experiments performed with smallpox vaccine of various ages and different methods of preparation the following conclusions were reached:

Vaccines shipped in a vacuum bottle from the New York City Health Department gave 100 per cent vesicle formation in the previously unvaccinated. Vaccines prepared according to the method of Noguchi showed rapid deterioration giving 4 per cent of failure on second passage, 50 per cent on fifteenth passage, and 90 per cent of failure on the twenty-ninth passage of a strain received from New York. Vaccines kept constantly on the ice except when in transit from the manufacturing laboratories gave a very low percentage of failure four, five, and six months after collection, while vaccines used within a month after collection gave 100 per cent of vesicle formation in the previously unvaccinated. Since temperature is so important a factor in the potency of smallpox vaccine, precautions should be taken to avoid a long period of transit except in vacuum bottles.

A properly cooled vaccine should give absolutely 100 per cent vesicle formation in the previously unvaccinated. Since the object of vaccination is to secure immunity, successful vaccination consists in producing either a primary vaccinia, a secondary vaccinia (vaccinoid), or a reaction of immunity. A reaction of immunity in a previously unvaccinated person indicates previous smallpox. A natural immune (provided such a person exists) would give no reaction of immunity. A vaccination certificate, therefore, should be an evidence of immunity, not a confession of failure.

UNIFORM VACCINATION TECHNIC.

The following technic is employed in all vaccinations. A vial containing 1.0 c.c. of smallpox vaccine (which is enough for 250 vaccinations) is opened, placed in cracked ice, and covered with a bell jar. The arm in the region of the deltoid insertion is washed with alcohol and dried with sterile cotton. A small chisel provided with a carbon steel point is sterilized by dipping in alcohol and flaming. Three circular scarifications each 2.0 mm. in diameter are made through the epidermis by rotating the chisel against the tightly drawn skin. A sterile toothpick is used to transfer a drop of smallpox vaccine from the containing vial to the three scarifications, and to rub it into the exposed derma. If tube vaccine is used it is expelled on the spots and rubbed in by means of the chisel. The site of the vaccination is then covered with a square of sterile gauze held in place by adhesive tapes on one of which is stamped the return date, five days subsequent to the day of vaccination.

The vaccinia produced by this technic follows a fairly constant course. In a group of about 300 vesicles measured on the fifth day, the minimum diameter was 3.5 mm., the average fell between 6.0 and 6.5 mm., the maximum was 9.0 mm., while 70 per cent were between 5.5 and 7.5 mm. The vesicle attains its maximum development on the eleventh day with an average measurement of 12.0 mm. Between the eleventh and fifteenth days the vesicle rapidly dries and shrinks to a brown scab which, in at least 60 per cent of the cases, becomes loose enough to be easily detached by the twentieth day. The areola surrounding the vesicle closely parallels its growth until the seventh day when it increases rapidly in size from an average of 10.0 mm. to an average of 40.0 mm. This sudden increase of the areola marks the rise of antibody formation, the vaccine organisms are digested, their toxic products give rise to the constitutional symptoms of fever and headache, while the change from vesicle to pustule marks the end of the vaccine colony. The areola rapidly shrinks to the size of the scab and gives color to the fresh scar which averages 11.0 mm. in diameter. This course is followed irrespective of the preparation of vaccine used or its bacterial content. Treating with antiseptics the vaccine vesicle produced by this method of vaccination does not decrease the area of redness or shorten the course of the vaccinia.

Vaccinias of long duration and marked severity result from improper vaccination technic. Given a smallpox vaccine of proven potency, we believe that

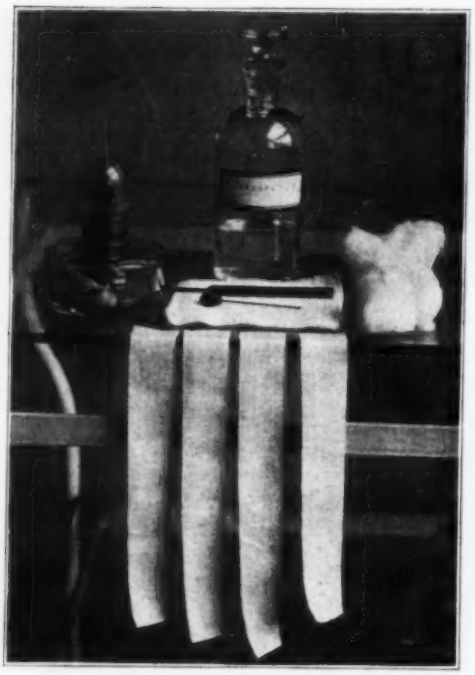

Fig. 7.—Equipment necessary for vaccination with ordinary tubed vaccine.

Fig. 8.—Cleansing the arm with alcohol and cotton.

Fig. 9.—Flaming the chisel which has been previously dipped in alcohol.

Fig. 10.—Scarification of the arm. Three circles of epidermis are removed exposing the derma.

Fig. 11.—Dropping the vaccine on the scarified spots.

Fig. 12.—Rubbing the vaccine into the scarifications.

Fig. 13.—Dressing of gauze and adhesive tapes. One tape bears the return date.

Fig. 14.—Cross-scarification. One of the causes of "bad" arms.

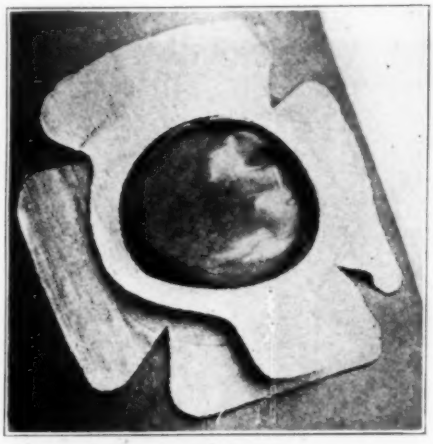

Fig. 15.—The vaccination shield. Another cause of "bad" arms.

Fig. 16.—Scar resulting from improper vaccination. Central necrosis, slough, infection, and a slowly healing ulcer. Patient thinks he is well protected because he had such a "hard take."

uniformly successful vaccinias, uncomplicated by secondary infections, may be secured by the use of small multiple scarifications. Since the vaccine colony grows only in unbroken skin, we wish to confine the growth to the smallest possible area for any one insertion. Cross-scarification leads to a crust which furnishes shelter for possible secondary invaders, the large rambling vesicle is easily broken and there is a tendency for central necrosis with a slowly healing ulcer. The results of insertion by single scratches are less constant than by the circular scarifications.

We believe that this method can only be improved by intradermal inoculation of a definite quantity of smallpox vaccine and we are working on this latter method at present.

REPORT ON PROPHYLACTIC VACCINATION WITH B. TYPHOSUS, B. PARATYPHOSUS A, AND B. PARATYPHOSUS B.*

By Wilburt C. Davison, M.D., Brooklyn, N. Y.

RECORDS of the Franco-German War, the Boer War,[1] the Russo-Japanese War[2] and the Spanish-American War[3] clearly show that the number of deaths from enteric fever in those wars was very large and closely approximated that due to wounds. The success of the work done by Wright, Russell,[3] Kabeshima[4] and others in regard to immunity against typhoid fever has been admirably demonstrated by its practical application in the armies of the United States, of Japan, and of all the countries now at war. It may be safely stated that since prophylactic vaccination against typhoid fever has been made more or less universal among armies, the losses from that disease have become practically negligible (that the records of prophylactic inoculation against typhoid in the Boer War is not more favorable is due to the fact that typhoid cases were not always distinguished from paratyphoid A and B).

During the present war, the paratyphoid infections have assumed the prominent place formerly held by typhoid. Official figures are not obtainable but conservatively stated there have been several thousand cases of fever due to B. paratyphosus A and B, while only a few hundred have been due to B. typhosus, the majority of the latter being in unvaccinated individuals. The relative frequency of B. paratyphosus A and B varies according to the location in which the epidemic arises. In India and along the Mexican border, B. paratyphosus A predominates; in Gallipoli and France, B. paratyphosus B is the more frequent.

The recent campaign of our troops in Texas and Mexico has also clearly indicated the efficacy of prophylactic typhoid vaccination; for the American Army receives as a routine three doses of typhoid vaccine of 500 million, 1,000 million, and 1,000 million bacilli respectively, at intervals of 7 to 14 days.

The very urgent need for similar protection against the paratyphoid infections is also seen, for while the number of cases of typhoid fever at the Mexican border was practically nil, there were hundreds of cases of paratyphoid. Captain Berry[5] reports that 21 per cent of the Fourteenth Regiment New York Infantry developed paratyphoid fever at some time during its three months on the Mexican border at Mission, Texas. Similar facts are shown in the studies at Camp Whitman in New York. The ease with which a paratyphoid epidemic spreads is demonstrated by the fact that twelve cases of paratyphoid fever were reported in the Seventy-first New York Regiment, ten to fifteen days after their one night camp at Mission, Texas, during the hike course, and fifty cases were found among their two hundred native laborers. The need for prophylactic paratyphoid vaccination is again demonstrated by the recent examinations for the detection of carriers among the men of the Fourteenth Regiment New York Infantry after its return from Texas. Four per cent of this regiment were found by Krumwiede and Kohn[6] to be carriers of B. paratyphosus A,

*Submitted to the National Research Council by Dr. John Howland, Medical Department Johns Hopkins University, Member of the Committee on Medicine and Hygiene.

and B. paratyphosus B, though there was no evidence of their having suffered from acute disease.

Prophylactic paratyphoid vaccination is practically the sole means of preventing such epidemics of this disease as occurred in France, Gallipoli, and along the Mexican border.

Castellani,[7] Cummins and Cumming,[8] Kabeshima,[4] Vincent, Dreyer,[9] Ainley Walker,[10] Davison,[11] and others have demonstrated the advantages of prophylactic vaccination with combined typhoid, paratyphoid A and paratyphoid B vaccines. Kabeshima has not only shown that simultaneous inoculation with a vaccine containing equal parts of B. typhosus, B. paratyphosus A, and B. paratyphosus B produced immunity against these three infections in man and animals but he also has proved that the reaction to this so-called "triple vaccine" was not more severe than that due to the typhoid vaccine alone.

One of the problems in relation to prophylactic vaccination against the enteric fevers is whether or not a better immunity against these infections is obtained by inoculating with the three microorganisms simultaneously or by immunizing with each bacillus separately by means of a succession of inoculations. It is in regard to these questions, as well as in regard to the course of immunity (as measured by agglutinins) that, at Dreyer's suggestion, I performed, in 1915 and 1916, under the supervision of Ainley Walker, a series of inoculations in a number of men and rabbits with vaccines prepared from cultures of one, two, or three of the bacilli (B. typhosus, B. paratyphosus A, and B. paratyphosus B), varying the doses and the order of their introduction in the successive immunizations of different groups of subjects (unpublished work). For instance, one group received the three varieties of bacilli simultaneously, the second group first received inoculations of B. typhosus, and after an interval of two months, inoculations of B. paratyphosus A, and B. paratyphosus B simultaneously, the third group first received inoculations of B. paratyphosus A, and B. paratyphosus B simultaneously, and after an interval of two months inoculations of B. typhosus. These experiments showed that when a *mixed "triple vaccine"* of B. typhosus and the paratyphoid bacilli was used, the immunity produced (as measured by the agglutinins) obtained for each of its constituent bacilli was *at least as good as and very often greater* than, that obtained against any one of these organisms when it is employed alone in the same dosage. Secondly, when single vaccines are employed in succession and the immunizations are carried out independently, the response is greater to that vaccine which is introduced first. To the later immunizations with other microorganisms the specific response is almost always less intense. It has associated with it, however, as a secondary result, the production of a new rise of variable extent in the agglutinin-titer of the first immunization; i. e., if the individual is immunized first with B. typhosus and later with B. paratyphosus A and B, the immunity (as measured by the agglutinins) is greatest for B. typhosus and less for B. paratyphosus A and B, and vice versa.

The results of these experiments have answered the objection raised by some that the inoculation of paratyphoid A and B bacilli simultaneously with B. typhosus, in triple vaccines, might reduce the typhoid immunity. If anything,

when B. paratyphosus A and B are inoculated simultaneously with B. typhosus, in triple vaccines, the titer of the typhoid agglutinins is raised.

In these experiments it was found that in man a triple vaccine made so that 1.0 c.c. contained 1,000 million B. typhosus, 500 million B. paratyphosus A, and 500 million B. paratyphosus B, 0.5 c.c. of which was inoculated subcutaneously, followed after an interval of 10 to 16 days by the inoculation of 1.0 c.c., gave excellent results.

These methods of triple vaccination were adopted by the British Army in January, 1916, and have been made compulsory. A third inoculation of 1.0 c.c. of triple vaccine after an interval of 10 to 16 days would, in all probability, be even more efficacious but the exigencies of the training and mobilization of troops have made this impractical in many instances and so it has not been adopted.

The reaction to this dosage was in every instance not more severe than that to the usual vaccination against B. typhosus alone, namely the inoculation of 500 million B. typhosus, followed after an interval of 7 to 14 days by 1,000 million B. typhosus.

Reactions to typhoid and triple vaccines are usually noticeable only in those individuals who have previously suffered from typhoid or paratyphoid fevers, or those individuals who react severely to such infections as "common colds." The local reaction in such individuals consists in redness at the point of inoculation and slight stiffness of the arm. The general reactions are a feeling of sleepiness, slight headache and rise of temperature and occasionally some general malaise. In the vast majority of individuals no local or general reactions are produced, especially if rest and freedom from arduous duty are granted to the individual on the morning following the vaccination.

It has been noted that certain typhoid vaccines are more liable to produce more severe reactions than others. The reason for this is as yet unknown but it is usually found that fresh vaccines made in accordance with the technic described later (which is essentially that of United States Army vaccines) do not cause more than a moderate reaction. Kilgore[12] has shown, in regard to typhoid vaccines, that the use of army vaccine and sensitized vaccine sediment (Gay and Claypoole)[13] if the preparations are fresh makes little if any difference in the average local and temperature reactions.

Kilgore and Meyer[14] state that in comparison with army vaccine, sensitized vaccine sediment produces small amounts of agglutinins but an equal amount of complement-fixing antibodies.

Garbat and Meyer[15] found the sera of rabbits immunized with sensitized vaccines were highly protective (more so than those of animals treated with non-sensitized preparations) and yet these potent sera contained but small amounts of agglutinins and complement-fixing antibodies.

However, it has not as yet been decisively demonstrated in man that sensitized vaccines have any great advantage over the army vaccine that has been usually used in this country and abroad.

The length of time during which this triple vaccination will provide immunity has not, as yet, been definitely determined, for a longer period than one

year. In all probability, however, immunity is present a more prolonged time and a two year interval before reinoculation is usually adopted.

Official figures for the number of cases of typhoid and paratyphoid fevers in the British Army since the introduction of prophylactic vaccination with "triple vaccine" are not obtainable, but one high authority,[16] in December, 1916, one year after the adoption of triple vaccination, stated that there were practically no cases of typhoid and paratyphoid in the British Army. The withdrawal from the Gallipoli Peninsula is not accountable for this sudden decrease, for the Salonica and Balkan campaigns are in a territory nearly as unhealthy.

The method of making triple vaccine adopted in our experiments was briefly as follows: stock cultures of B. typhosus, B. paratyphosus A, and B. paratyphosus B were grown on agar, either in tubes or Roux bottles, for 24 hours at 37° C. The growth was then washed off with normal saline to which 0.5 per cent of phenol had been added. Each bacillary suspension was killed by heating in a water bath at 45° C. for 30 minutes and then gradually raising the temperature to 56° C. and maintaining it at that level for one hour. The suspensions were then proved sterile (both culturally and by inoculation into rabbits) and then counted (either by Wright's blood film method or by a Zeiss bacterial counting chamber). The suspensions were then diluted and mixed so that 1.0 c.c. contained 1,000 million B. typhosus, 500 million B. paratyphosus A and 500 million B. paratyphosus B. This triple vaccine was again proved sterile (culturally and by rabbit inoculation).

Prophylactic vaccination against B. typhosus, B. paratyphosus A, and B. paratyphosus B will eliminate practically all cases of infection with these three organisms; a few cases will still occur, the majority, as shown by experience, because some of the troops have failed to be vaccinated, through "conscientious objections" or through oversight; and a certain minimum in whom the vaccination does not produce sufficient immunity.

To differentiate these cases, which are practically always mild, from other pyrexias, an accurate quantitative method of serologic diagnosis must be used.* The agglutination test when quantitatively performed by an accurate technic[17] can undoubtedly be made to give as valuable results in inoculated as in uninoculated persons; for although it is true that a single positive Widal reaction, even when done quantitatively, in the case of a person suspected of typhoid or paratyphoid fever who has previously been inoculated with triple vaccine, may be of no value, yet by recording the highest dilution in which marked agglutination occurs (that is, the titer of agglutination of the patient's serum) and repeating this quantitative determination once or twice at intervals of four days, one can readily arrive at a correct diagnosis by noting whether or not any appreciable alteration occurs in the titer of the patient's serum at the successive examinations (that is, noting whether the agglutinating power of the serum has risen or fallen during the period of observation). For one can state with confidence that if either a marked rise or a marked fall in the agglutination titer has taken place, the person is suffering from active typhoid or paratyphoid infection, since it has

*For the first 8 days a blood culture is a reliable method of diagnosis, but after this period, a positive blood culture is rare and positive stool and urine cultures are usually not obtained in more than 60 per cent of the cases.

been shown[18] that in active typhoid and paratyphoid infections the agglutination titer of the serum increases for about the first three weeks (Dreyer), after which it falls, at first rapidly, and then much more gradually toward its original level. The rate of fall progressively diminishes, so that after about two months, successive observations made at short intervals, will probably exhibit no appreciable change.

The agglutination technic by means of which this accurate analysis has been rendered possible is the quantitative macroscopic method introduced by Dreyer[19] in 1904, of which this brief description issued from the Department of Pathology, University of Oxford, is appended.

DIRECTIONS.

USE OF STANDARD AGGLUTINABLE CULTURES IN TESTING THE AGGLUTINATING POWER OF A SERUM.

I. *Technic.*—Take a stand* containing 15 agglutination tubes in 3 rows of 5 each, and a dilution tube. (See note on size of agglutination tubes on p. 614.)

With the proper dropping pipette measure out into the dilution tube 54 drops of normal saline solution, 0.85 per cent sodium chloride, in distilled water (where the water supply is pure, tap-water can be used instead of saline solution) by means of gentle pressure on the teat.

Wash the pipette with distilled water.

Dry out the pipette with successive quantities of absolute alcohol, followed by successive quantities of ether, and get rid of the ether.

Take up the serum to be tested into the dried pipette. Measure out 6 drops of the serum into the dilution tube already containing the 54 drops of saline solution, thus obtaining a dilution of 1 in 10. Mix thoroughly.

Carefully wash out the pipette.

With the pipette measure out into each row of tubes as follows:

Number of tube.	Drops of Normal Saline Solution.	Drops of Serum Dilution 1 in 10.	
1	0	10	to each tube in row 1 add 15 drops of *B. typhosus* Standard Agglutinable Culture.
2	5	5	to each tube in row 2 add 15 drops of *B. paratyphosus A* Standard Agglutinable Culture.
3	8	2	
4	9	1	to each tube in row 3 add 15 drops of *B. paratyphosus B* Standard Agglutinable Culture.
5	10	0	

At each stage of the procedure the pipette is carefully washed and dried out with successive quantities of absolute alcohol followed by successive quantitives of ether.

Shake each tube thoroughly in order from right to left, i.e., beginning each row with the highest dilution.

Place the stand for 2 hours in a water-bath at 50°-55° C. (*not* in dry air).

In Tube 1 of each row the serum acts in a dilution of 1 in 25.
" 2 " " " 1 in 50.
" 3 " " " 1 in 125.
" 4 " " " 1 in 250.

Tube 5 containing no serum is control against spontaneous agglutination.

If the limit of agglutination is not reached within this series, higher dilutions are followed out in a similar manner.

Thus, for example, 57 drops of normal saline solution plus 3 drops of a 1 in 10

*Stands, dropping pipettes, agglutination tubes, etc., can be obtained from Messrs. Baird and Tatlock, Hatton Garden, E. C., London. If the larger agglutination tubes are used the quantities of saline, serum and culture are measured in tenths of a c.c. in the same proportions as the drops.

serum dilution will give a serum dilution of 1 in 200, and, using the same quantities as before, one has the serum acting in dilutions of 1 in 500, 1 in 1000, 1 in 2500, and 1 in 5000. And similarly for higher dilutions.

The tubes are examined after 2 hours at 50°-55° C. followed by 15 minutes standing at room temperature. The reading is taken by comparing each tube in succession with the control tube, and is preferably made by means of artificial light against a black background. If daylight is used, the tubes inspected should be partly shadowed by passing a finger up and down behind them.

The highest dilution in which marked agglutination (without sedimentation) can be detected by the naked eye is *standard agglutination*. But owing to the rate at which the dilution increases in the series of tubes employed it will commonly happen that no tube in the series exhibits *standard agglutination*. If this be so it will be found in looking along the series that while one tube shows strong agglutination with sedimentation the next succeeding tube shows no agglutination at all or only a trace. In such cases standard agglutination lies approximately midway between the two dilutions.

Should a more precise determination of the limits of agglutination be required, it can be obtained by using a stand of 12 tubes with the series of quantities given in the table contained in the directions for Preparation and Standardization of Agglutinable Cultures.

(If the stand is left at the room temperature, 16 to 24 hours must be allowed before the reading is taken, but the reaction is not then so sharply defined. In this case the highest dilution in which a definite flocculent sedimentation appears corresponds approximately to standard agglutination.) The same is true for 8 hours in an incubator at 37° C.

When the standard degree of agglutination (*"standard agglutination"*) occurs with Standard Agglutinable Culture in a serum dilution of 1 in x, then x divided by the figure given on the label of the Standard Agglutinable Culture employed gives the number of *"standard agglutinin units"** contained in 1 c.c. of the serum examined.

Thus if standard agglutination occurs in a dilution of 1 in 1000 and the number on the label is 2.5, then $\frac{1000}{2.5}$, i.e., 400, is the number of Standard Agglutinin Units contained in 1 c.c. of the serum examined.

For uniformity and simplicity in recording results they should be expressed in *standard agglutinin units.*

2. *Diagnosis.*—A. In non-inoculated persons who have not had typhoid (or paratyphoid) fever, agglutination in a dilution of 1 in 25 justifies a strong suspicion of typhoid (or paratyphoid) infection. But the test must be applied again in the course of a few days to ascertain whether there is any change in the titer of agglutination. Marked agglutination in a dilution of 1 in 50 or more is (nearly always) diagnostic of active typhoid (or paratyphoid) infection.

A *non-inoculated* "carrier" will normally show no important change in the titer of his serum on repeated examination at short intervals.

B. Inoculated persons if quite recently inoculated will usually show a high titer of specific agglutination. A rapid rise in titer sets in within two to four days of inoculation. This is followed by a fall at first rapid, but subsequently becoming very slow, so that a relatively high titer is maintained for a long period (even for years). During this period examinations made at intervals of a few days give practically identical readings.

It follows that in the case of inoculated persons the diagnosis of active typhoid (or paratyphoid) infection will require two or more successive examinations of the serum.

(*a*) If the individual is suffering from active *typhoid* infection his titer of typhoid agglutination will exhibit the usual rise and subsequent regular fall seen in non-inoculated subjects, but starting from and returning towards the higher base line of inoculated persons.

(*b*) If the individual is suffering from active *paratyphoid* infection one of three things may occur as regards his *typhoid* agglutination titer namely:

1. No appreciable change may occur in the titer of typhoid agglutination.
2. A relatively slight rise may occur, followed by a fall towards the former level.
3. A marked rise may occur synchronous with the rise in paratyphoid agglutination titer, and subsequently followed by the usual fall towards the former level.

Meanwhile the titer of *paratyphoid* agglutination runs the normal course of rapid

rise to a maximum (usually exceeding the maximum typhoid titer) followed by a fall, at first rapid and then slower as already described for typhoid subjects, and falling *below* the persistent base line of typhoid agglutination of inoculated persons.

C. In the case of mixed infections whether in inoculated or noninoculated persons the agglutinin curves for the different infecting organisms are usually not synchronous, and they pursue their ordinary course independently of each other.

**Note.*—The *standard agglutinin unit* is that amount of agglutinating serum which when made up to 1 c.c. volume with normal saline solution causes standard agglutination on being mixed with 1.5 c.c. of a particular standard agglutinable culture and maintained at 55° C. for 2 hours in a water-bath followed by 15 minutes at the room temperature.

From the Department of Pathology, University of Oxford, on behalf of the Medical Research Committee.

In Great Britain and Denmark this technic has now very largely superseded the older microscopic method. Its application and the use of the standardized agglutinable cultures,[20] which have been issued since July, 1916, on behalf of the Medical Research Committee (England) to British military and naval hospitals, have rendered it a simple matter to determine accurately the degree to which the serum of any given individual will agglutinate the Bacillus typhosus or the paratyphoid bacilli.* A description of the mode of preparation and standardization of these cultures is sent out from the Oxford laboratory in the following form:

Preparation.—The bacillus (B. typhosus, B. paratyphosus, etc.) is grown for twenty-four hours at 37° C. in ordinary veal peptone bouillon in large Erlenmeyer flasks partly filled (1 liter of bouillon in a 1½ liter flask).

The bouillon is titrated against phenolphthalein, and two-thirds of that amount of sodium hydrate which would render it neutral to phenolphthalein is added before the final boiling and filtration.

Before use the flasks of bouillon are sterilized in the autoclave at 115° C. for *not more* than fifteen minutes, and are then tested for sterility by incubation at 37° C. for forty-eight hours.

They are inoculated with a few drops each from a twenty to twenty-four hour old bouillon culture of the bacillus (B. typhosus or B. paratyphosus, etc.).

The culture used should be one which has been subcultivated daily in bouillon for one or two weeks (or longer). This continued subcultivation has the effect of increasing its agglutinability and diminishing any tendency to spontaneous agglutination.

At the end of twenty to twenty-four hours' growth at 37° C., the flasks are well shaken, and to each is added 0.1 per cent (1 c.c. per liter) of commercial (40 per cent) formalin. They are again shaken and placed in a cold chamber in the dark at about 2° C.

At intervals on the same day and on subsequent days for four or five days the flasks are again thoroughly shaken and *replaced at once in the cold chamber.*

After three or four days they will be found to be absolutely sterilized. Should it happen that the bacterial suspension is not entirely homogeneous it may be shaken for some hours in a mechanical shaker, or may finally be filtered through sterile cotton-wool.

Standardization.—The process of standardization consists (A) in making up the killed culture to an opacity as nearly as possible identical with that of the Standard Agglutinable Culture, (B) in measuring its agglutinability as compared with the Standard Agglutinable Culture by the use of Standard Serum.

A. The killed culture is diluted to the required degree with normal saline solution to which has been added 0.1 per cent of commercial (40 per cent) formalin.

A. *Details of Method.*[22]—*Apparatus.*—Almost any variety of dwarf test tube or agglutination tube may be used. It is essential to have at hand a fairly large supply of tubes, so that a selection may be made of tubes of practically equal internal diameter. The tubes must be quite clean and their surface must be free from scratches. When the

*Any laboratory can be readily equipped to make and issue in large quantities these standard agglutinable cultures of typhoid and paratyphoid bacilli. They may also be obtained by writing to the Standards Department, Department of Pathology, Museum, Oxford, England.

greatest possible accuracy is desired, it is best to use the larger* form of tube and to measure the fluids with a 2.0 c.c. pipette, accurately graduated in hundredths of a cubic centimeter. But for ordinary purposes the small** agglutination tubes may quite well be employed, and the dilutions made with a dropping pipette. In this latter method there is a certain error due to the difference in size of the drops of 0.85 per cent NaCl solution and of culture. About 17 to 18 drops of culture are equal to 15 drops of saline. But in dealing with small volumes of fluid, it is open to doubt whether measurements with a small graduated pipette are any more accurate than this.

Technic.—(For the smaller tubes the quantities of saline, serum and culture are measured in drops; for the larger tubes in tenths of a c.c.) Two sets of eleven gauged tubes are placed in a suitable stand. Into the tubes of each set the different fluids are measured as follows:

Tube	Saline	Culture
1	0	20
2	4	16
3	8	12
4	10	10
5	12	8
		Culture diluted 1 in 2
6	6	14
7	8	12
8	10	10
9	12	8
10	14	6
11	16	4

This series of dilutions is similar to that described for the serum dilution in the standardization of agglutinable cultures, only multiplied by two. It will be seen that the range of dilutions in the series is from 1/1 to 1/10, and that the progressively diminishing quantities of culture are always made up to the same volume with saline. The mixtures in the tubes are well shaken with the finger over the mouth of the tube, and the readings are then taken.

Readings.—Artificial light and a black background are essential. It has been our custom to use a frosted electric bulb with a large green shade, and some pieces of dull black boarding or paper placed on the table below the lamp and propped up behind it. A darkened room, though not absolutely necessary, adds considerably to the ease and accuracy of the readings. The lamp shade is inclined so that the bulb is just hidden from the eye, and the tubes to be compared are held up against the edge of the shade. Before reading, the outside of the tubes must be wiped with the moistened corner of a glass-cloth and then polished with the dry part of the cloth. From one series a tube is chosen at random which we call the "standard" tube for the first reading. With this the tubes of the other series are compared one after the other until one is found which matches it in turbidity. It will frequently be found that there is no tube in the second series which gives an exact match, but that there is one tube which is a little more turbid while the next lower is a little less. In this case the standard tube is recorded as lying between those two tubes of the other series.

Moreover it is usually possible to judge whether it is nearer to one than to the other. If so, we make a mark or marks under the number of the tube to which it is nearer. Five or six readings should be made in this manner, taking a number of tubes in succession as standard. It is well to read in both directions; i.e., to take tubes from each series as standard. Now, since we know the dilution of the culture in every tube, it needs only a simple calculation to arrive at the relative turbidity of the two cultures. Records of typical routine estimations are given in Table I. Moreover, twelve tubes may be used with advantage instead of eleven.

Thus: Culture (c.c.) 1.0, 0.8, 0.6, 0.5, 0.4, 0.35, 0.3, 0.25, 0.2, 0.17, 0.13, 0.10. Water (c.c.) 0.0, 0.2, 0.4, 0.5, 0.6, 0.65, 0.7, 0.75, 0.8, 0.83, 0.87, 0.9. The range of dilution is the same as before; and the only difference is that the series is fuller by one tube at the right-hand end, which renders readings in this neighborhood more accurate.

*A thin-walled dwarf test tube about 7.0 cm. long and 0.95 to 1.0 cm. in diameter.

**A smaller "agglutination tube" 5.7 to 5.8 cm. in length, having an internal diameter of 5.5 to 6.2 mm.

Moderate variations in the color-intensity of the fluids do not appreciably affect the accuracy of the readings, for the progressive dilution renders such differences negligible at an early point in the series. If one of the fluids under comparison is so thick that the range of dilutions described proves insufficient to allow a comparison with the other fluid; then a further series of tubes must be set up in precisely the same manner, but using the thicker fluid diluted (1 in 10, or 1 in 20) instead of at full strength.

In practice it is often convenient to make a rough estimate of the relative thickness of the fluids before proceeding to the complete test. This is easily done by taking a small quantity of the two fluids in two tubes of equal caliber, and diluting the thicker fluid until the two show approximately equal turbidity. From this rough test it is easy to make an approximate calculation of the relative thickness of the fluids. If one fluid appears to be four or five times thicker than the other, it is best to dilute it suitably before putting up the test series.

TABLE I.

ESTIMATION OF RELATIVE TURBIDITIES OF FLUIDS A AND B.

A is a stock standard culture
B is a fresh, undiluted, formalized broth culture
Technic: graduated 2.0 c.c. pipette and large tubes

Readings		Actual quantities of culture in the matched tubes		Ratio $\frac{\text{quantity of A}}{\text{quantity of B}}$	Deviation from mean in per cent
Series A Tube	Series B Tube	A	B		
5 matches	9	0.40 c.c	0.20 c.c.	2.00	0.50
2 "	5	0.80 c.c	0.40 c.c.	2.00	0.50
6 "	10	0.35 c.c	0.17 c.c.	2.06	2.49
3 "	7	0.60 c.c	0.30 c.c.	2.00	0.50
7 "	10-11	0.30 c.c	0.15 c.c.	2.00	0.50
				Mean 2.01.	Mean error, 0.90%.

Calculation for diluting B to the same turbidity as A:
1.0 c.c. of B = 2.01 c.c. of A.
Therefore, to each 1.0 c.c. of B add 1.01 c.c. of diluting fluid.

B. To measure the agglutinability of the killed culture thus diluted, proceed as follows:

Take two stands and place twelve agglutination tubes in each. Prepare (1) a dilution of standard agglutinating serum of such strength that each cubic centimeter contains from 4 to 8 standard agglutinin units, and from this prepare (2) a second dilution of half that strength.

With the pipette measure out

Drops of normal saline solution.		Serum dilution.	
Into Tube 1 of each stand	0	10 drops* of dilution	1
" " 2 " " "	2	8 " " "	
" " 3 " " "	4	6 " " "	
" " 4 " " "	5	5 " " "	
" " 5 " " "	6	4 " " "	
" " 6 " " "	3	7 " " "	2
" " 7 " " "	4	6 " " "	
" " 8 " " "	5	5 " " "	
" " 9 " " "	6	4 " " "	
" " 10 " " "	7	3 " " "	
" " 11 " " "	8	2 " " "	
" " 12 " " "	10	0 " " "	

(To each tube of one stand is added 15 drops of *Standard Agglutinable Culture*, and to each tube of the other stand 15 drops of the *killed culture* under standardization.)

*If the larger tubes are used these quantities are measured in tenths of a c.c. in the same proportion as the drops.

At each stage of the procedure the pipette is carefully washed and dried out with successive quantities of absolute alcohol followed by successive quantities of ether.

The stands are placed for two hours in a water bath at 50° to 55° C., then allowed to stand for fifteen minutes at room temperature and a reading subsequently taken by selecting in the series made with standard agglutinable culture the tube which exhibits standard agglutination (the highest dilution in which marked agglutination, without sedimentation, can be detected by the naked eye), and ascertaining which tube in the other series shows the same degree of agglutination. Should the tube be the same in each series, the agglutinability of the killed culture is clearly equal to that of the standard. If not the same, the degree of agglutinability of the killed culture is now readily determined.

Thus suppose that Tube 5 in the Standard series corresponds to Tube 2 in the other series. The Standard Agglutinable Culture is twice as agglutinable as the killed culture under standardization, since only half the quantity of serum has been required to agglutinate it to the same degree.

Hence, if any given serum presented for examination is found to agglutinate this particular killed culture in a dilution of, say, 1 to 500, then 500 multiplied by 2 and divided by the figure given on the label of the Standard Agglutinable Culture is the number of Standard Agglutinin Units in 1 c.c. of the serum examined.

Or, again, if the killed culture were, say, 1.3 times as agglutinable as the Standard Agglutinable Culture, then in the same example as above, 500 divided by 1.3 and again divided by the figure given on the label of the Standard Agglutinable Culture is the number of Standard Agglutinin Units in 1 c.c. of the serum examined.

As a matter of routine, quantitative agglutination tests are made simultaneously against B. typhosus, B. paratyphosus A, and B. paratyphosus B, and the highest dilutions in which marked agglutination without sedimentation occurs are recorded. Since, wherever triple inoculation has been made a routine practice, it is found that about 99 per cent of the inoculated individuals give a positive agglutination reaction (Widal) against B. typhosus, B. paratyphosus A, and B. paratyphosus B for at least a year, therefore a second specimen of blood must be taken four or five days later and the test repeated. If the highest dilution in which marked typhoid or paratyphoid A or B agglutination occurs on the second occasion is approximately the same as that found on the first occasion, the case is not one of typhoid or paratyphoid A or B fever, and the agglutination is due to the triple inoculation alone. For it has been shown that after about the third month of antityphoid and paratyphoid A and B inoculation, the titer of the agglutination due to inoculation is practically stationary if examined at intervals of only a few days, and that tests made at intervals of even weeks will show only a small gradual decrease in titer.[21] If, on the other hand, the highest dilution of marked agglutination for any of the bacilli (typhoid and paratyphoid A and B) in the second test is either markedly higher or markedly lower than at the first examination, one can state that the case is one of active infection with the bacillus whose titer showed the alteration, i. e., either B. typhosus or B. paratyphosus A, or B. paratyphosus B.

CONCLUSIONS.

1. Prophylaxis against typhoid and paratyphoid A and B is absolutely necessary for armies operating in the United States and Europe.

2. Mixed "triple" vaccine containing in 1.0 c.c.; 1000 million B. typhosus, 500 million B. paratyphosus A and 500 million B. paratyphosus B; 0.5 c.c. of which is injected subcutaneously, followed at an interval of 7 to 16 days by the injection of 1.0 c.c. at a further interval of 7 to 16 days is advisable.

3. Accurate quantitative agglutination tests with standard agglutinable cultures made at four day intervals is the best method of diagnosis of typhoid and paratyphoid infection in inoculated individuals.

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THE SIGNIFICANCE OF LAMBLIA INTESTINALIS IN STOOL EXAMINATIONS*

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THAT certain protozoal parasites have a definite pathogenicity in some forms of intestinal disturbances is acknowledged by practically all clinicians. The role played by the flagellate, Lamblia intestinalis, is still an open question if one is to judge by the diversity of opinion expressed in the literature on the subject. In the writings of Braun, Park and Williams, Barker, Besson, Allbutt and Rolleston, and many others, it is tersely stated that they are nonpathogenic. Other writers, among them Emerson, Stiles, Rodenwaldt, MacNeal, and Neveu-Lemaire, consider that their part as an etiologic factor in chronic diarrhea is not entirely settled, but that they may have much to do in prolonging symptoms when the disease is once established. On the other hand, Daniels, Brumpt, and Stitt each ascribe a pathogenic role to these organisms, the last mentioned author stating that they are "responsible for a chronic and intractable diarrhea, an infection only minor in importance to amebic infection."

We have not attempted to review the literature fully, but may state that in recent articles on the subject Lamblia intestinalis has been considered pathogenic by the following authors: Du Bois and Toro, Wenyon, Russell, Kennedy and Rosewarne, Porter, Fantham, Fantham and Porter, and Mathis.

In tropical regions this parasite is encountered very frequently in stool examinations. In our country, especially in northern latitudes, it is not found commonly. The cysts are easily recognized, and when present are found in great numbers if the patient has had the usual preparation for examination for endamebas, i.e., one-half to one ounce of salts before breakfast. It is quite usual, when cysts are present, to find free, motile forms the next morning after the patient has taken a second dose of salts.

In stool examinations made for more than 6,000 patients during the past six years, we have found this parasite in only a trifle more than 1 per cent of the cases. The patients came from all parts of the United States, but chiefly from the northern states; three were from Canada.

This report, then, is based on 66 cases in which Lamblia intestinalis has been found in stool examinations. In 41 persons this was the only organism that might account for the complaint. In the remaining 25 cases there were other organisms, or pathologic conditions which presumably, in some instances at least, accounted for the complaint.

In studying the histories of these cases we find there is apparently no syndrome which is diagnostic without a stool examination. A history of preceding, or present diarrhea, usually without blood or mucus, often with most or all of the stools in the early morning hours, with rumbling and rolling in the intestines, indefinite pain slightly more to the right side, an "all in" feeling, and nervous indigestion are all suggestive symptoms, but in no way diagnostic. After

*From the Mayo Clinic, Rochester, Minn.

the initial attack of diarrhea, constant looseness, alternate constipation and diarrhea, or constipation may remain.

As lamblias have been held to be nonpathogenic by most observers, no attention has been paid to their presence in the stools, and little or no treatment has been directed toward their removal. Possibly for this reason many of our patients give long histories. Nineteen of the series of 41 patients gave a history extending over five years, 11 of them having a constant looseness or severe diarrhea. Eighteen of the series had constant looseness varying from 3 to 20 stools daily; 8 others had spells of diarrhea; 3 had normal stools; and the remainder were constipated.

As the lamblias inhabit the upper intestinal tract, we would not expect free blood in the stools or much inflammation in the colon. Six of the 41 patients had seen some free blood (one of these had bleeding piles), 18 expressly stated

Photomicrograph of Lamblia intestinalis.

that no blood had been passed, and in the other cases no mention of blood was recorded. In the 25 cases in which there were complications, 5 patients had noted blood and 13 had not.

Proctoscopic examinations were made for 10 of the 41 patients. Six had normal bowels, 3 a slight proctitis and 1 a diffuse colitis without ulceration. Of 5 patients in the series of 25 who had proctoscopic examinations, 2 had normal bowels, 1 a mild colitis, and 2 ulceration due to amebas.

Twelve of the series with lamblias alone had examinations of the stomach. In 9 the acids were normal, and in 3 there was absence of hydrochloric acid. Six patients had barium roentgenographic examinations of the stomach, and in all the findings were negative. Blood examinations were made in the majority of the cases, but showed no change in the eosinophiles and in only two patients of the larger series was the hemoglobin reduced (45 per cent).

Since the symptoms definitely indicated some trouble and in many cases were of long duration, and since lamblias were not considered pathogenic, other

organs than the intestines were suspected to be the site of the trouble. Ten of the 41 patients were operated on, and 3 of the other group of 25. One of the 10 had carcinoma of the rectum and was operated on five years after we first found the lamblias. One was operated on for enlarged spleen which later turned out to be a lymphosarcoma. Five had appendicectomies; 4 were not helped; the fifth had had a history of several definite attacks of appendicitis, and was relieved of that trouble. On 2 cholecystectomies were done without relief; and in 1 the gall bladder was the surgical objective, but was found normal. In this case a pericolitis was considered the cause of the trouble. Three other patients had surgical diagnoses—1 a diagnosis of appendicitis, 1 of cholecystitis, and 1 of duodenal ulcer—but were not operated on.

Of the three operations in the second group, 1 appendicectomy gave no relief, 1 cholecystectomy and appendicectomy gave no relief, and 1 appendicectomy on a patient with a history of ten definite attacks of appendicitis gave relief.

Nine, only, of the 41 patients were treated in the clinic, and these for a short time only. Thymol and methylene blue were used. Thymol, given in 6 instances, relieved the symptoms temporarily, and removed lamblias from the stools in 4 cases. However, only one stool test was made after treatment in each instance. In 1 case a severe diarrhea was stopped and the patient gained 27 pounds, but four months later the symptoms recurred. In another case the diarrhea stopped and the hemoglobin rose from 45 per cent to 68 per cent in one month. Methylene blue was of benefit in only 1 case.

In 10 of the cases in which amebas were a complication, treatment consisted of emetin, ipecac and coal oil enemas, the lamblias being disregarded. Five of these were symptomatically relieved, though in 1 case there was a recurrence in six weeks. In none did this treatment remove the lamblias. In 1 instance in which both lamblias and amebas were present, the patient was treated with thymol and coal oil enemas; both organisms were removed, and the patient was relieved of the diarrhea.

Thus, it is likely that when the pathogenicity of these parasites has been disregarded, the diagnosis and treatment of many patients has not been correct; and that when Lamblia intestinalis has been considered the cause of the condition, and treatment has been instituted against it, better results have been obtained.

CONCLUSIONS.

1. Lamblia intestinalis should probably be considered a pathogenic parasite.
2. We have obtained no definite syndrome in our series.
3. The removal of lamblias is difficult.
4. The best results have been obtained from thymol medication.

TWENTY-FIVE CASES OF LAMBLIA INTESTINALIS WITH OTHER COMPLICATIONS.

Complications.	*Cases.*
Endameba histolytica	9
Endameba histolytica and active pulmonary tuberculosis	1
Endameba histolytica and history of passing recent tapeworm and hookworm	1
Actively motile ameba (not diagnosed)	1
Nonmotile ameba	2

Encysted Endameba coli	2
Endameba coli	2
Ova of tenia	1
Active pulmonary tuberculosis	4
(1, with tuberculous peritonitis)	
(1, tubercle bacilli found in stools)	
(1, pulmonary symptoms came eighteen months after abdominal symptoms)	
Pernicious anemia	2

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CHLOROMA: WITH REPORT OF A CASE*

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IN recent years, there has been extensive literature presented upon the group of diseases associated with the blood-forming organs. This has, undoubtedly, been stimulated by the confusion of classification and insufficient knowledge of their etiological factors. Considerable evidence has been accumulated to show that many of the specially named blood diseases are closely related to each other, even to such an extent that some observers believe that they are transmutable. Cases closely simulating the one recently reported by Warfield and Kristjansen which showed the transition of lymphosarcoma to acute lymphatic leukemia and thence to Hodgkin's disease lend much to this latter view.

Chloroma is very closely related to the acute leukemias and probably should be considered under them. The term chloroma was first used by King in 1853, when he applied it to a case of greenish tumors of the head, coming under his observation four years previously. The first report of this condition is attributed to Allan Burns, who described it in 1823. He, however, did not attempt to name the condition. His case was one of greenish yellow tumor masses occurring about the bones of the head. Dock reported the seventeenth case to which reference is found in the literature in 1893 and briefly reviewed the sixteen previous cases. Since then, sixty-nine cases, classified as chloromata, have been reported.

From the cases which have been described it would seem that the term is not entirely appropriate if the condition is to be regarded as a separate pathological entity, inasmuch as cases have occurred in which all of the clinical and pathological features ascribed to chloroma have been present except for the green color, and others where only some of the nodules, glands or blood clots showed the green color at autopsy. "Nonchloromatous leukemia" has at times been applied to the former type of cases, but it also would appear to be a misnomer. Sternberg, in his classification of the diseases of the hematopoietic organs, describes under hyperplasia of lymphoid tissues with leukemic blood and having tumors originating in various situations and invading tissues, leukosarcoma. If the tumor masses of such a condition are of green color, the name chloroleukosarcoma is applied. Likewise such a condition with leukemia of myelogenous origin is named chloromyelosarcoma. These terms seem appropriate if they are used to describe a form of acute leukemia with an origin in the bone marrow, in which the cells are so malignant in type as to erode the bones to form tumor masses upon their surfaces, some or all of these masses presenting a greenish color. In most of the cases described as chloroma previous to that of Klein and Steinhaus, reported in 1904, the cell type was thought to be of lymphocytic origin and the condition was classified by various writers as a form of lymphatic leukemia, lymphosarcoma or a transitional stage between them. Since then, however, the majority of authors have classified the cell as of myelogenous origin and the condition as a form of acute myelogenous

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leukemia. Both Treadgold and Burgess convey the idea that it is probably always of myelogenous origin. The increase in the proportion of reported cases of myelogenous origin is probably due, as suggested by Burgess, to the more thorough examination of the cells both morphologically and biologically, as by the oxydase reaction, which he thinks has shown that certain of the cases formerly classed as acute lymphatic leukemia must be considered as myelogenous. Forman and Warren recently reported a case of myeloma, many of the cells of which, by ordinary staining methods, seemed to be plasma cells. The cells, however, contained oxydase granules, showing them to be of the myelogenous series. Without such examination this case probably would have been called plasmoma.

That the condition is always due to a primary involvement of the bone marrow is shown by the fact that in practically all cases in which the bone marrow was examined it was found altered; that the location of the tumor masses attached to the bones is always found in the short bones and those of the skull, in the marrow of which the hematopoietic function is still active; that the cells evidently have grown directly through the bones to form the masses, as the surfaces of the bones are eroded and the masses fairly well attached; and that the cells in a number of cases have been definitely proved to be myeloblasts and myelocytes since their protoplasm either showed granules by differential staining methods or oxydase granules upon application of the oxydase reaction.

The blood picture of an acute leukemia, concomitant with chloroma shows the same type of cell which is predominant in the green tumor, as well as in sections of the bone marrow. Burgess states that the highest leucocyte count was 1,880,000 per cubic mm. In a few cases no increase of white blood cells was noted and for these forms the term "aleukemic chloroma" or "chloromatous pseudoleukemia" was suggested. However, there is not sufficient evidence to introduce this terminology as indicating a distinct type of disease.

Although the terminology is confusing, it would seem that when the term chloroma is used, a type of leukemia is referred to in which there is the formation of green masses of immature white blood cells. The leukemia is probably always myelogenous in character and is due to a primary hyperplasia of the red bone marrow. The marrow cells develop to such a degree that they erode and grow through the bone to form green tumor masses upon its surface, as well as evidence metastatic power to enter the blood stream and lodge in distant organs, where they may multiply. Some or all of the cell collections, whether seen as tumors upon the surfaces of certain bones, as nodules in the organs, or occurring in the blood clots, bone marrow or glands, may show the greenish color at autopsy. Treadgold says, "Since it is usual in chloroma for a varying proportion of the lesions to show no green color, it is theoretically possible to get a fairly acute type of myeloblastic leukemia, bearing a general resemblance to chloroma, but without the green lesions."

The clinical and pathological features of most of the cases are similar. It occurs more frequently in children, particularly males. When not occurring in children, the most frequent age incidence is after the fourth decade. The patient generally complains of weakness, anemia, pain due to pressure of tumorous masses, and a tendency to hemorrhage. Exophthalmos is frequent, due to

green tumor formation in the orbital fossæ. The disease runs an acute course from weeks to months and so far as known is invariably fatal. Leukemia is present. At necropsy, the chief findings are flattened greenish tumors over the inner surface of the bones of the head, vertebræ, sternum, or ribs. The bony surface beneath is eroded and to it the tumor is attached. The marrow spaces are increased in size and the bony trabeculæ rarefied. Tumor nodules may be found in the various organs and some of the blood clots may have the greenish color. An absolute diagnosis cannot be made, except by encountering the green color in the tissues. This has been done in several cases during life by finding the presence of green nodules subcutaneously. Attempts to diagnose by the symptoms and location of masses is only conjecture as the same may occur in acute leukemia, lymphosarcoma and myeloma. The diagnosis was made in a case reported by Butler upon the following points; age 11 years, acute leukemia, exophthalmos and right-sided deafness. The exophthalmos in this case, contrary to expectation, was found not due to the size of the orbital tumor mass but to vascular engorgement. This shows how easy it is for one to err in the diagnosis of certain manifestations.

While the report of the two cases by Gould and Le Wald is noteworthy, yet in the absence of autopsies and definite leukemia, their classification as chloroma is uncertain. Their diagnosis before death was made upon age (children), exophthalmos, periosteal and medullary changes of the bones, glandular enlargement, and microscopical picture of glands removed from the neck. No leukemia was present in the first case and the white blood count in the second case was only 16,500. Such a count could be present in lymphosarcoma or many infections.

The following case was under the service of Dr. J. A. Lichty to whom I am indebted for the clinical notes:

The patient, an adult male, age 36 years, was admitted to the Mercy Hospital, November 16, 1915. He complained of weakness and pain in the right chest and beneath the sternum. His family history was negative. There was previous history of measles, mumps, whooping cough, diphtheria, typhoid fever, malaria, lues and Neisser infection. His luetic infection had occurred seven years previously and for one year he had received mercury by mouth and inunctions. Cardiorespiratory and digestive systems were negative. On admission, physical examination showed a well developed male, not acutely ill. A moderate amount of anemia was present. The pupils were equal, regular in outline, symmetrical and reacted to light and accommodation. No exophthalmos or other ocular signs. Some purulent exudate was present over the left tonsil. Very little glandular enlargement present. The thorax showed a distinct depression over the right apex and subclavicular region and very little rotation of ribs of the right side on respiration. The percussion note over the right apex, right subclavicular region, and region lateral and inferior to the right scapula was impaired. No ascites. The liver was palpable at the costal margin. The spleen seemed enlarged to percussion but not palpable. Wassermann reaction for lues was negative.

HEMATOLOGICAL EXAMINATION.

	Nov. 17, 1915.	Nov. 20, 1915.	Nov. 22, 1915.	Nov. 26, 1915.	Nov. 30, 1915.	Dec. 7, 1915.
Hemoglobin	30			20	15	
Red blood cells	1,632,000			872,000	600,000	675,000
White blood cells	53,000	44,000	43,000	45,200	138,200	320,000
Myeloblasts	73	76		82	77	
Polymorphonuclears	9	11		11	7	
Transitionals	1				2	
Large lymphocytes	11	6		2	3	
Small lymphocytes	6	7		5	11	

Blood Smears.—The blood smears were stained by Wright's method. The white blood cells were greatly increased in relative proportion to the red blood cells. A number of nucleated reds were observed. The leucocytes varied in size from 8 to 21 μ in diameter. All of these cells, even the forms appearing as small lymphocytes, were pale staining. Sometimes the nuclei of the small lymphocytes showed vacuolation, often giving a rosette arrangement. The large mononuclear forms classified as myeloblasts were round or oval and had very pale, large, single nuclei, which occupied the greater portion of the cell so that only a relatively small rim of cytoplasm appeared at the periphery. The nuclei did not appear reticulated. The cytoplasm was faintly basophilic and slightly darker in staining quality than the nuclei. At the time when these smears were made there was no mention of specific granules in these cells. It is probable, however, that they were somewhat different from true myeloblasts, as a new examination of the smears one year later showed the presence of a small percentage of both eosinophilic and basophilic myelocytes. The granules of the polymorphonuclear leucocytes were very faint and could not be made out with the stain (Wright's) used. Possibly with better staining methods for granules these could have been demonstrated in some of the cells termed myeloblasts. Polymorphonuclear leucocytes were few in number. During life no examination was made for oxydase granules in the cells of fresh blood films. This was attempted some time after death, but the films were too old to demonstrate the reaction.

The clinical diagnosis was "myeloblastic leukemia." The patient died three weeks after admission to the hospital. During this time the white blood count as shown above increased gradually from 53,000 to 320,000 cells per cubic mm. An autopsy was performed 4 hours after death by Drs. W. W. G. Maclachlan and J. W. Fredette.

AUTOPSY.

The body was that of an adult male measuring 160 cm. in length. The body was fairly well developed, but poorly nourished. The skin surfaces were very pale. There was no rigor mortis and only slight lividity on the dependent parts. The pupils were equal and moderate in size. Scattered over both arms, upper left thorax and abdomen, were a number of tiny petechial hemorrhages. The thorax was well formed. The abdomen was slightly flattened. There were no scars on the penis. The anus was normal. There was slight edema of the ankles.

Thorax.—On both sides there were some fibrous adhesions over the posterior surface of the lungs and also at the apices. About 100 c.c. of clear straw-colored fluid was present in each pleural sac. The pericardial sac contained a clear straw-colored fluid in a quantity of about 100 c.c. In the fat over the pericardium were a number of petechial hemorrhages. An occasional hemorrhage was also noted on the parietal and visceral pericardium. Lying opposite the left apex of the lung beneath the parietal pleura and over the inner surface of the ribs and also on the bodies of the thoracic vertebræ there was a thick, quite green, firm, smooth tissue, forming a layer, which, although well attached to the bony structures underneath, could be peeled away leaving evidences of an eroded underlying bone. This tissue when removed measured 5x4x1.3 cm. Its pleural surface adjacent to the lung showed carbon pigmentation to a very marked degree, otherwise it was green. On section, the cut surface had a glassy, smooth appearance. There were some fibrous tags running between this tissue and the apex of the lung. The green color faded gradually after the removal of the tissue. The pleura covering the ribs showed many tiny petechial hemorrhages.

Left Lung.—Weight 630 gm. The posterior surface and the apex of the upper lobe showed a few dense fibrous adhesions. The lung was quite black in color showing much anthracosis. It felt moderately firm but crepitated in most places. Section through the lower lobe showed a cut surface, fairly dry, of grayish pink color, and with very prominent nodules of anthracotic pigment. The upper lobe was a little lighter in color and somewhat moister. At the apex, opposite the fibrous adhesions, was a small area of fibrosis in the lung substance. There was no calcification nor caseation noted here. The bronchi were free. The peribronchial glands showed marked anthracosis, but no evidence of tuberculosis.

Right Lung.—Weight 800 gm. There were some fibrous adhesions over the posterior portion of the lower lobe and on the diaphragmatic surface. The lung felt firm but crepitated in most places. The lower lobe, on section, showed a somewhat moist pinkish gray surface, glassy throughout, with numerous nodular areas of anthracosis. Petechial hemorrhages were also seen in the lung substance. There was no consolidation. The upper lobe showed the same characters, and fluid could be expressed from its substance.

The edema in this lobe was more marked than in the other lobes or in the other lung. The bronchi showed much frothy fluid but their lining was not congested.

Heart.—Weight 415 gm. The heart contained a large quantity of soft chicken fat clot of a more gray color than that usually seen. This clot was present in all chambers of the heart. The heart valves had very little noticeable change. The mitral valve showed a slight amount of fibrous thickening. Scattered over the surface of the heart were petechial hemorrhages. Shining through the pericardium, over the ventricles, one could distinctly discern yellow fatty stippling in the heart muscle. When the heart was opened this stippling was beautifully seen shining through the endocardium, particularly in the papillary muscles and columnæ carnæ. The myocardium was pale yellow in color. On section through the myocardium of the left ventricle the diffuse yellow stippling was well brought out. The muscle was of fair consistency. The F. O. was closed. There was no evidence of fatty change in the endocardium, but petechial hemorrhages were particularly noticeable under and in the endocardium of the auricles. The coronary arteries showed patent lumina with no diminution in size or change in the lining. The aorta was thin and elastic, and showed but little yellow intimal change.

Abdomen.—The fatty layer was well marked and of a pale yellow color. The recti muscles were bright red and quite thick. The great omentum was fatty and covered the upper coils of the small intestine. The intestines were somewhat collapsed. There was no fluid in the abdominal cavity. The appendix was small showing a slight constriction about the middle due to a band of fibrous adhesions. The organ lay behind the lower end of the cecum and pointed upwards. The mesenteric glands were not enlarged. Some were slightly reddened, suggestive of the hemolymph gland type. Throughout the mesentery were many tiny petechial hemorrhages. The liver reached to the costal margin. The diaphragm arched to the right 5th rib and 5th space on the left.

Stomach.—The stomach was of good size. There was a pale smooth healthy looking lining. The pylorus was small and normal.

Intestines.—The small intestine showed nothing of note and was healthy. The lymphoid follicles were not enlarged. There was a good deal of sacculation of the wall of the large bowel, but no diverticula were noted.

Liver.—Weight 1825 gm. Measured 29x17x9 cm. The margin was sharp. The surface was smooth and shiny. Through it yellowish stippling could be seen. On section the liver was of a chocolate color, swollen and glassy. The outlines of the lobules were not distinct. Scattered thickly over the cut surface of the liver was a fine pinhead sized, yellowish white stippling. These areas were not regular in size. They appeared to lie in the outer portion of the lobule. The whole cut surface was somewhat nutmeg in appearance, although no central zone of congestion in the liver lobule was present. The liver surface was more brownish than the typical nutmeg type. In consistency the liver was about normal. The gall bladder was small and contained about 50 c.c. of tarry bile. The wall was thin and pigmented by bile on its inner surface.

Pancreas.—Measured 19x3x2 cm. The organ was firm. The cut surface showed grayish lobules which were normal.

Spleen.—Weight 190 gm. Measured 13x8.5x3.5 cm. The notches were quite distinct and the capsule appeared a little wrinkled and was covered by a geographical mottling of white lines suggesting lymphatic vessels. On section through the spleen, the organ appeared fairly firm, and little substance could be scraped off with the knife. The cut surface showed very prominent Malpighian tufts. The trabeculæ were but little evident. At two points a slightly darker spleen substance was observed. These areas were roughly triangular, but not clearly outlined. They were not suggestive of true infarction. In size these two areas were 1 cm. across the base and 1.5 cm. in depth.

Left Kidney.—Weight 235 gm. Measured 12x7x5 cm. The capsule peeled readily leaving a slightly granular and extremely pale cortex. In the capsule there were a few petechial hemorrhages. On the cortex there was a stippling of congested vessels. Scattered over the surface of the cortex were a number (about 12) of round slightly raised bodies 2 mm. in diameter. On section through these bodies they were seen to extend into the cortex about 2 mm. They had a light green color, were perfectly smooth, clearly outlined, and of the consistency of the cortex of the kidney. On section through the kidney the cut surface presented marked pallor, decided swelling of the cortex, and an indefinite marking. The glomeruli were poorly shown. In the deep cortex several green nodules similar to those described on the surface were to be seen. There was also some diffuse, whitish streaking and stippling which gave the cortex a rather granular appearance. The

fine capillaries of the cortex stood out prominently. About the pelvis there was considerable fat. The pelvis and ureters were healthy.

Right Kidney.—Weight 200 gm. Measured 5x5x3 cm. The capsule peeled fairly readily leaving very pale cortex with fine stippling of congested vessels on the surface. Similar small slightly green oval bodies were noted on the cortex. The cut surface of the kidney showed the same appearance as the left, with pale, swollen cortex and indistinct marking. There was one more decided green nodule in the cortex of the same size as the others. The deep cortex was similarly thickened, showing yellowish streaking and stippling as seen on the opposite side. The pelvis and ureters appeared free. There was a moderate amount of fat about the pelvis.

Adrenals.—The adrenals were of good size and showed marked yellow cortical layers and small white medullary portions.

Bladder.—The bladder was of good size. The wall was smooth and pale. The ureters were patent. The prostate was small and pale in color. The cut surface was glassy.

Testicles.—The testicles were of normal size. The cut surface showed a brown, soft character. The tubules could be easily pulled out.

Bone Marrow.—The bone marrow of the femur was pale red in color and appeared very moist. The color was darker and more intense than the normal marrow. In the marrow a large amount of cancellous bone tissue was found.

MICROSCOPICAL EXAMINATION.

Green Tumor Mass in Pleura.—The section showed a very cellular structure. The stroma was loose and appeared in most places as a fine reticulum. In other places it was denser and showed numerous collagen fibers. The only blood vessels seen in this stroma were capillaries. The predominant cell was rather large, about 12 micra in diameter, and mononuclear. The shape was round or slightly oval. The cytoplasm (stained with eosin and methylene blue) was neutrophilic and contained no specific granules. However, with higher magnification the cytoplasm was seen to have a ground glass appearance. A cell membrane was present. This cell had a large round, oval or sometimes irregular nucleus, vesicular in character, with a definite nuclear membrane and rather scattered, coarse, deeply staining chromatin granules or masses. Exclusive of these scattered chromatin masses, the nucleus was pale. Cells with smaller deeply staining nuclei and a relatively great abundance of neutrophilic cytoplasm were rather common. There was also present a number of polymorphonuclear leucocytes and eosinophilic and basophilic myelocytes. Very few typical lymphocytes were seen. Clear nuclear division was not seen, but binucleated forms were present. One of the sections showed the tumor cells to have entirely surrounded several large nerves. Fat globules lay between the tumor cells in many places. Another section of the tumor showed the cells to have surrounded a spinal ganglion. Some of the cells had invaded the substance of the ganglion.

Bone Marrow.—Section of the bone marrow showed a moderate congestion and a slight amount of marrow fat. The bone marrow cells were nearly all of the types described in the section of the tumor. About one per cent of the cells were lymphocytes.

Lung.—The section showed a deposit of granular precipitate throughout the alveoli associated with considerable red cell infiltration. Some of the alveoli contained fibrin. The walls of the alveoli were thickened, due to the dilated blood capillaries filled with tumor cells lying in the alveoli and surrounded by masses of fibrin and red blood cells. A moderate number of polymorphonuclear leucocytes were also noted. There was considerable anthracotic pigment about the vessels and bronchi. One of the large pulmonary vessels contained thrombi, made up of tumor cells, blood and fibrin. Occasionally, endothelial cells containing anthracotic granules were seen lying behind the alveoli. The tumor cells seen in the blood vessels, when stained with hematoxylin and eosin, had much the appearance of plasma cells.

Heart.—The section showed a rather loose, somewhat edematous interstitial tissue, which caused a moderate separation of the muscle fibers. The fibers were not very clear in their staining. Their transverse striations were not well seen, though the longitudinal ones were much better brought out. The nuclei were clear. There was very little pigment about the nuclei. The muscle fibers further had a finely granular, vacuolated appearance. There was no cellular infiltration present, but in the capillaries an unusual number of cells were seen which were similar to those seen in the blood smears.

Liver.—Section of the liver showed some congestion. The general outline of the

liver lobules was a little altered by the presence of large areas of necrosis. These necroses lay mostly in the central zone, although many reached into the more peripheral parts of the lobule. In the necrotic areas, there was an infiltration of cells similar to the type of cells seen in the tumor. A few polymorphonuclear leucocytic cells were seen. An infiltration of the tumor cells was also seen around the portal systems. Under high magnification, the indistinct shells of the liver cells could be still made out. In other portions of the section, liver cells of the central zone appeared very watery, showing but a cell membrane and nucleus. The rest of the liver tissue, particularly the peripheral zone, was relatively healthy, although the cells were swollen and granular. Much bile pigment was present in the liver cells throughout. The sinusoids were dilated and filled with granular precipitate. The bile capillaries were also dilated. In the sinusoids were many tumor cell elements of the usual type. Lymphoid cells were also seen in moderate numbers about the portal systems. Fat vacuoles were numerous in the central zones of the lobules.

Spleen.—The section of the spleen showed no congestion. The follicles were clear in outline. The trabeculæ were not large or numerous. In some of the follicles, masses of hyaline were to be observed. The sinuses were packed with mononuclear cells, similar to those of the green tumor mass. Through the section much blood pigment was noted within the endothelial cells. A hyaline change was observed in the walls of many of the vessels. The pulp was filled with cells similar in type to those found in the blood stream elsewhere, and also to those noted in the tumor mass. The polymorphonuclear leucocytes were few in number. The stroma in the spleen was not increased. In one portion of another section of the spleen was seen a large area surrounded by fibrous tissue. In the center of this area, there was a homogeneous pale blue staining material. Surrounding the area of fibrosis, there was a rather thick wall of tumor cells.

Kidney.—The section of the kidney was taken through one of the green nodules. There was also a good deal of uninvolved kidney tissue present in the section. The tubules showed granular looking cells with a moderate amount of debris in the lumen. The cells were quite irregular in outline and some were devoid of nuclei. The glomeruli were not changed, except in their capillaries, where a few abnormal cells were seen. In the capillaries of the interstitial tissue, tumor cells were numerous. The green nodule was seen to be a mass of tumor cells, which, in the center, had entirely replaced the tubular structure, while towards the periphery the necrotic remains of tubules were still to be seen. Near the outer margin of the tumor mass, the tubules were separated, but still living. The outline of the tumor nodule was fairly regular and quite devoid of any capsule, while the kidney tissue adjacent appeared normal. The cells of the nodule were the same as those noted in the tumor elsewhere. Another section of the kidney showed a little connective tissue increase following the course of the blood vessels. An occasional glomerulus was fibrosed.

Sections of the pancreas, prostate and adrenal showed normal characters.

Postmortem Bacteriology.—Cultures of the heart blood showed streptococcus pyogenes.

Oxydase Reaction.—Examination for oxydase granules by the Schultz method was made with the tissues one year after death. No oxydase granules were seen by this method due to the age of the materials. However, with Graham's method, which will stain the granules after a long period of time has elapsed, the majority of the tumor cells gave a positive oxydase reaction.

Anatomical Diagnosis.—Chloroma; myeloblastic leukemia; hyperplasia of bone marrow; chloroma of ribs, vertebræ, pleura and kidney; fatty degeneration of heart and liver; old bilateral pleural adhesions; petechial hemorrhages of skin, pleura, pericardium, endocardium, heart, mesentery, and lung; fibrosis of lung (right apex); anthracosis of lung; central necrosis of liver; edema of liver; enlarged spleen (slight).

Resume of Case.—The patient was a man of 36 years, complaining of weakness and pain in the chest. He had no exophthalmos, or glandular enlargement. Repeated examination of the blood showed a progressive leukemia. Clinical diagnosis was myeloblastic leukemia. At autopsy there was found a greenish tumor mass attached to the inner surface of the ribs and bodies of the vertebræ which when peeled out left evidence of erosion of the underlying bone. Green nodules were found in the cortex of the kidney. The bone marrow of the femur was hyperplastic. Petechial hemorrhages were present in the skin over the arms, thorax, and abdomen, beneath the pericardium and endocardium, in the lung, mesentery, and kidney capsule. The predominant cells of the blood were similar to those of the bone marrow and of the green tumors. Oxydase granules were present in these predominant cells showing them to be of myelogenous origin.

DISCUSSION.

The study of this case contributes nothing to the etiology of the condition. As with other tumors, nothing is known of the causative factor. The presence of streptococcus pyogenes in the heart blood at autopsy is indicative of a terminal infection coincident with the low bodily resistance present just before death and has no etiological bearing. Sternberg cites eleven cases of acute myeloblastic leukemia, nine of which showed organisms either in the spleen, heart blood, or bone marrow. Seven of these cases showed streptococci, six times alone and once combined with staphylococcus aureus; one showed staphylococcus aureus alone and one staphylococcus aureus with streptococcus and influenza bacilli. He concluded that leukemic blood could be obtained in various diseases and that many cases which had been diagnosed as leukemia were but the reactions of the blood-forming organs to the original infections, and that the above cases of "so-called acute myelogenous leukemia" were nothing other than acute general infections which proceeded with myelogenous and myeloid metaplasia and were to be differentiated from the true and genuine leukemias. He offered this in explanation why some of the so-called "acute leukemias" can be cured while the true leukemias always have a fatal ending. But when it is considered that Strauch, in a series of two thousand postmortem blood cultures found an organism in 50.1 per cent of the cases and Fredette in one hundred and nineteen blood cultures taken immediately after death found organisms in one-third of the cases, it is quite probable that some of Sternberg's infections were terminal ones and were not the cause of the leukemic blood pictures. Rother found the same blood picture in a case of acute miliary tuberculosis as in myelogenous leukemia, but there is no way by which we can exclude a myelogenous leukemia as being coexistent with the acute miliary tuberculosis. Gans also recognized a similarity sometimes existing between the blood picture caused by infectious diseases and the blood picture present in spontaneous disease of the myelogenous system.

Although the case under discussion occurred in an adult, the great majority of cases are seen in children. This is sometimes explained as due to the fact, that in childhood there exists a relatively greater amount of red marrow and the hematopoietic organs are more active. But why should not myelogenous leukemia also occur more often in children, if this is the cause? The cell in chloroma is often more embryonal than that found in the ordinary type of acute leukemia. This, and the fact that the cell appears more malignant than that of acute leukemia may have something to do with its occurrence in children.

Color of Chloroma.—The greenish color of the chloromatous mass over the ribs and vertebræ faded rapidly on exposure to light and air. Portions of this mass kept in formalin regained its greenish color upon the following day, when immersed in hydrogen peroxide solution. After a period of one year in formalin, the tissue did not regain its former color after a like treatment. The same occurred with regard to the color of the green nodules of the kidney. The green color did not go into solution in either the formalin or the Zenker's solutions in which tissue was preserved. Nearly all observers noted the rapid fading of the color on exposure to light and air, and that the color cannot be preserved by means of chemical solutions, nor can it be recovered by ordinary

aqueous solvents. Exceptions, however, are found to these usual findings. Dock and Warthin in their case (1904) noticed a temporary increase in the depth of color after the removal of the tissue. Alt found that the tissues in his case retained their dark green color in formol solution and the fluid itself was a dirty green color. Risel reported that the color was not preserved in Kaiserling's solution. Burgess observed that tissue preserved in 10 per cent formalin for three years regained its former color in hydrogen peroxide and that in one instance the color was preserved several weeks in a strong solution of sodium bicarbonate, and to a very slight degree in certain of the tissues fixed in formalin and in Kaiserling's solution. Potassium hydroxide restored the color slightly to formalin treated tissue. Walls and Goldsmith say that the color in their case persisted in Kaiserling's solution for four and one-half years. Ayers found that on placing the green tumor in alcohol, the color disappeared entirely within twenty-four hours, but a specimen kept within a corked bottle retained its greenish color. The bone marrow of the tibia in this case showed a redder marrow than is usually present. The character of the tissue of the ribs and vertebræ, where one would expect more change present, was not examined. Burgess thought the redness of the bone marrow in his case was due to congestion of the capillaries. About seven cases have been reported which showed green marrow in some of the bones.

Various theories have been offered for the green color. The variety of the color reactions reported has led some observers to the opinion that it is not always due to the same pigment. By some it has been attributed to iron or fatty substances, but neither of these has been demonstrated as the cause. Reynolds held that the greenish color was of the nature of a fatty acid combined with iron. Walls and Goldsmith concluded that the color was not due to a foreign pigment such as iron, and believed that the consensus of opinion indicates that it is something inherent in the cells of the tumor masses, much the same as the chlorophyl of green plants. Schmidt suggests that the color might be due to the shape of the cells. Risel, Lubarsch and Weinberger demonstrated that the color was not of bacterial origin. Treadgold found that the green color is not present from the beginning, but that it makes its first appearance in the early lesions, and, therefore, that cellular degeneration plays a part in the formation. This degeneration may be due to the age of growth or facilitated by the toxemia. He suggests that possibly a degeneration of the granules or the perigranular protoplasm of these cells, or an abortive attempt to form granules, is the real source of the color, aided by broken down products of hemoglobin.

Sections of the green mass were stained for hemosiderin by Nishimura's modification of the Perls reaction and for fat by Sudan III, but neither were found to be present in the chloroma cells. Risel demonstrated hemosiderin granules in the tumor cells, but did not ascribe the color to these. Lubarsch was unable to find pigment granules in either sections or fresh preparations. Dock (1893) noticed abundant highly refractile granules in the cells of frozen sections. Huber and Chiari found granules which they thought were fatty in nature. Von Recklinghausen believed the color to be of a parenchymatous nature similar to the color of old pus. In the case of Dock and Warthin (1904) no pigment granules were present. Osmic acid and Sudan III tests were nega-

tive. That the color is not a postmortem finding is proved by the green lesions noted during life. Dock suggested that "the peculiar color was simply an exaggeration of the greenish tint so common in the blood clots and neoplasms of leukemia."

At the suggestion of Dr. W. W. G. Maclachlan, examination was made of the white blood cells from a case of myelogenous leukemia at the Mercy Hospital. The leucocytic count was 1,000,000 per cubic mm. Over ninety-nine per cent of these cells showed oxydase granules. Blood was drawn into a test tube with sufficient sodium citrate solution to prevent clotting, and the test tube was corked and allowed to stand twenty-four hours. When examined the buffy coat of white blood cells had a yellow color with a faint greenish tint. After standing for five days in the ice box, this layer showed a decided greenish color and the serum also had a slight greenish tinge. Upon shaking the tube and mixing the red blood cells and the leucocytes, the greenish color was entirely obliterated by the red. The formation of this distinct greenish color in the leucocytes on standing five days, emphasizes Treadgold's theory that the color is not present from the beginning, but that cellular degeneration plays a part. If this greenish color has any relation to that of chloroma, one might expect it where myelocytes and myeloblasts are present *en masse*, as in the cellular tumors of chloroma, which has but little stroma and is practically devoid of red blood cells. Chloroma would then be a type of myelogenous leukemia where the myelogenous cells appeared in dense collections. However, the green color observed in the myelocytes of the blood did not fade on exposure, nor did it deepen on addition of oxidizing reagents. That these two greenish colors may have a relation seems possible, but evidently they are not identical. A similar examination was made of the blood from a case of lymphatic leukemia where ninety-five per cent of the cells were lymphocytes. No green color whatever developed in the layer of white blood cells in this case.

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THE THYMUS—A SUMMARY*

By Paul G. Woolley, M.D., Cincinnati, Ohio.

At an early stage of development of the human embryo, a series of pouches (branchial) are formed in connection with the pharynx. These all resemble one another except as regards their size. The anterior are larger, and from them the primitive tympanic cavity and the palatine tonsils arise. The posterior (the third and fourth) give rise to the thymus and the parathyroids.

The thymus in an early stage is a paired organ of epithelial origin, and in young fetuses (50 mm.) it appears as a closed vesicle lined with high cubical epithelium. During later growth and differentiation the small cells and cell nests appear, and cortex and medulla are formed. In its mature state the thymus has taken on a distinct lymphoid appearance, its masses of cells of lymphoid character enclosing varying numbers of cell nests which are known as Hassall's corpuscles. These are somewhat similar, in appearance, to epithelial pearls, which are the result of the concentric, overlapping arrangement of the flattened epithelial cells.

That these corpuscular cells are truly epithelial there seems to be no doubt, but there is no general unanimity concerning the other, so-called thymus, cells. By some they are presumed to be immigrated cells (thymus lymphocytes); by others, more especially by Stöhr,[1] they are looked upon as modified epithelial cells. Comparative and theoretic considerations undoubtedly speak in favor of their epithelial nature.[2] If this be true, that the thymus is permanently a true epithelial organ, then the typical tumors which originate in the thymus are indeed anomalous, for they have distinctly sarcomatous characteristics. Yet there are certain biologic facts that seem to speak for the lymphoid nature of the thymus cells. One of the effects of the action of the roentgen rays upon the thymus is disintegration of the lymphocyte-like cells of the organ. During this process, lymphocytolytic substances are produced which, if injected into other animals (peritoneally), result in lysis of the lymphocytes in the appendix and mesenteric lymph nodes.[3]

The thymus, beginning its active growth before birth, continues growing for some time—up to the time of puberty, it is said—after which period involution occurs. Its period of growth ends therefore with the beginnings of the ascendancy of the sexual glands. There is here the suggestion of a certain antagonism between the two sets of glands. But for one reason or another, involution may be hastened or delayed. It may be hastened by bad conditions of health, and delayed or slowed by good conditions. Severe illness, especially the infections of childhood, are apt to be succeeded by precocious involution.[4] This apparent relationship between thymus physiology and the general state of the organism led Sahli to speak of the gland as the measure of the state of nutrition. With precocious involution of the thymus there seems to be no precocious development of the sexual glands—nor the reverse.

The thymus is believed by many to be an important internal secretory or-

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gan, which exercises important powers over growth. It is possible that it is able to stimulate growth even though it seems to lack the power of exciting differentiation. Gudernatsch's[5] work indicates that it may be able to suppress differentiation. It is believed that it exerts an inhibitory action upon the sexual glands, the adrenals (especially the cortex), the thyroid, spleen, and pancreas, but these actions are not clearly demonstrated, and seem rather to be based upon coincidences than upon exact experimental results. Changes in the thymus are associated with such syndromes as status thymico-lymphaticus, exophthalmic goiter, dwarfism, achondroplasia, acromegaly, and certain types of precocious adipose states. As a matter of fact, variations in the size and microscopic appearance of the thymus are associated with so many morbid states, and so little has been accomplished by experimental work on it, that very little can be said definitely regarding its physiologic relationships. Our own experience is that there are no definite lesions or changes in the thymus that are associated with other organic or general physiologic abnormalities. This, it would seem, is a minority report. Consensus of opinion is in the opposite direction.

Since the first attempt at thymectomy by Restelli, in 1845, and the publication of Friedleben's experiments, in 1858, a large number of workers have studied the behavior of animals after incomplete and complete operative removal of the thymus. For the most part, until very recent times, all have reported that extirpation of this gland has no more than indefinite and transient after-effects. In 1898, Calzolari noted that the thymus undergoes hypertrophy after castration (rabbits), and J. Henderson called attention to the fact that in cattle castration is followed by increased growth and delayed thymus involution. Noel Paton, using guinea pigs, confirmed Henderson's observations, and added that if thymectomy is done before puberty, the testicles undergo unusually rapid evolution. It has also been remarked that the thymus is larger in capons than in cocks. Additional evidence of interrelationships between other glands and the thymus are offered in the observations of Tandler and Gross, that the latter gland is abnormally persistent in eunuchs; that splenectomy is followed by hypertrophy of the thymus (Mattei); that hyperplasia of the pancreas is apt to follow thymectomy (Mattei); that the thymus is commonly large in hypopituitarism; that it is increased in size after hypophysectomy (Cushing); that persistence of the thymus is fairly constant in acromegaly; that hypertrophy of the adrenals, both cortex and medulla, follows thymectomy (Klose, Mattei, Halsted); that excision of the adrenals is followed by hyperplasia of the thymus; that the use of adrenal extracts produces thymus involution (Wastenson, Boignet); that after thyroidectomy the thymus atrophies; and that after thymectomy, there is an increase of thyroid activity.[6] These data seem to suggest that the thymus is antagonistic to most every other ductless gland, which seems unreasonable. It is supposed that when these changes do not occur it is because accessory thymus tissue exists.

The most striking results of extirpation of the thymus have been obtained by using young animals. After the age of puberty, removal produces less obvious change. The reports chronicle very remarkable skeletal effects, largely in the direction of delayed development. Appearances suggestive of those of spontaneous rickets were reported by Basch,[7] who used young puppies in his

experiments. He noticed osseous changes, beginning two to three weeks after operation, which seemed to indicate deficient or delayed ossification, resulting in increased ease of fracture and increased flexibility. At points of fracture, the callus was deficient in lime salts. With these changes there were also nervous manifestations, some of which were peripheral (hyperirritability) and some central (decreased intelligence). Klose[8] practically duplicated Basch's experiments but used very young puppies (10 to 20 days old), and watched them for longer periods. He observed that after a period of about two weeks, the animals showed increasing adiposity, polyphagia, and loss of muscular activity, following which stage came a third, during which the animals lost weight rapidly, and also lost muscular and bony strength, but retained their appetites. At the same time they lost all psychic power, and finally, in the course of 3 to 14 months, died in coma. The bones from such animals showed extreme modifications which are characteristic of osteoporosis, and which included cyst formation, irregular thickening of the epiphyseal cartilages and deficient linear growths. Fractures which were more easily than normally produced, were healed by fibrous union. The bones showed a loss of about 35 per cent of calcium salts. Klose's explanation is that thymus removal takes away the locus of nuclein synthesis, and that this results in an acidosis (nucleic acid) which causes the removal of lime salts from the bones and at the same time produces an edema of the brain. He also assumes that the spleen assumes the thymic function after involution of the latter, a conception which is supported by the association of hypertrophy of the two organs in status thymico-lymphaticus and by the fact that x-ray, allowed to act upon the thymus alone, leads to a decrease in size of thymus and spleen.[9] The work of Klose, Klose and Vogt, Mattei, and others, points then to the fact that thymus removal produces important general changes in metabolism. Growth is delayed, ossification is slowed, the bones become soft and easily fractured, and healing of fractures takes place slowly and frequently by fibrous union. Also associated with these skeletal changes, a cachexia thymopriva develops after an uncertain latent period and is characterized by lassitude, muscular weakness, emaciation and increased stimulability of the peripheral nerves. Criticism of certain interpretations of the results following thymectomy are based upon the possibility of the removal of parathyroids with the thymus.

Pappenheimer's[10] observations have led him to believe that while rachitic changes do occur in white rats (with which he worked), these are not due to thymectomy, but to spontaneous disease. He was not able to confirm the results of Klose and Magnini. Nordmann[11] believes that the thymus is not an essential organ, and that its removal in young dogs has no important effects, although Flesch[12] has produced evidence that the organ is essential to the life of young rats.

The whole outcome of the work up to the present time seems to be as Wiesel says, that our knowledge of the function of the thymus and of its relationships to other glands is still deficient. One may be permitted to believe that the thymus may be a ductless gland which has a close relation to bone metabolism, to the chromaffin tissue and to the sexual glands.

The thymus is implicated in three well isolated clinical entities beside rachi-

tis; namely, status thymico-lymphaticus (mors thymica), exophthalmic goiter, and Addison's disease. Changes in the gland are also associated with various infections, certain cachectic states, with states of precocious adiposity, with dwarfism, and with certain forms of achondroplasia. In acromegaly it is fairly constantly persistent. The relationships which exist between the thymus and other glands is variously interpreted. Hammer divides all the ductless glands into two groups according to their action as excitors or depressors of the thymus. Others see little evidence of definite relationships.

STATUS THYMICO-LYMPHATICUS.

It has been noticed from time to time, especially since the reports of Paltauf, that apparently healthy individuals, who have died suddenly and unaccountably during anesthesia, or after falling into the water, or after apparently unimportant traumata, have possessed an enlarged, perhaps hyperplastic thymus. The suspected relations between the enlarged gland and death is expressed in the term mors thymica. It has also been noticed that, in these cases, the lymphoid tissue generally is hyperplastic. In typical cases, not only the peripheral lymph glands are large, but the whole lymphoid tissue of the intestinal tract as well appears remarkably hyperplastic. The spleen also is enlarged. Hence the term lymphatism, or status lymphaticus. In some cases only the lymphoid tissue is hyperplastic; in other cases, the thymus alone is affected; often both. In many such cases there is, beside the glandular condition, also evidence of defective physical development, for which condition, in prethymic times, the expression lymphatic disposition or temperament was used. Typical evidences of outspoken physical incompleteness are shown in a certain infantile appearance characterized by obesity, infantile genitals, and defective growth of the hair, especially the pubic.

Not uncommonly the cause of death in status thymico-lymphaticus is believed to be due to the enlarged thymus, almost filling the upper opening of the thorax, resting upon the great vessels, and the cardiac auricles; and hemmed in by the manubrium sterni and the upper ribs, which becomes acutely congested and compresses the organs about it (including the trachea), and so causes rapid death. In less extreme circumstances it leads to dyspnea which disappears when the organ becomes less swollen or after it undergoes involution. It is known that the x-ray is able to cause atrophy of the thymus and that it is very effective in preventing attacks of thymic asthma, as has been shown by Friedlander. The facts that x-ray treatments reduce the size of the thymus and that the effects of the treatments are very prompt, indicate that there is at least some basis in the physical cause of mors thymica; and, moreover, if the latent period that exists between operations and symptoms be actually due to the presence of thymus substances in the body, then it would seem that there should also be a very distinct latent period between x-ray exposure and relief—which does not seem to be true.

It has also been assumed that the cause of the complex status thymico-lymphaticus is an overfunctioning of the gland, and that under these circumstances there is an excessive amount of secretion given to the system. There seems to be as little proof of this as one might expect from our knowledge of

the physiology of the gland. N. Paton believes that the syndrome is essentially a pluriglandular one in which several glands participate, and that in the absence of the secretions of these glands and because of general imperfect development, death is more easily produced by traumata which would otherwise be ineffective. Wiesel also considers the complex the expression of a general constitutional anomaly—a general tissue weakness. This generally anomalous condition is suggested by the frequence of abnormalities in persons, the subjects of status lymphaticus. The more frequent lesions found in connection with it are hypoplasia of the adrenal medulla, narrowing of the aorta, congenital heart lesions, enlargement of the thyroid, and of the spleen and lymph glands. Also, in children, these lesions are apt to be the lesions of rickets plus a large thymus.

It has been said that primary and secondary morbus Addisoni are almost regularly associated with status thymico-lymphaticus.[13] It seems entirely possible that this is true of the Addisonian complex when the chromaffin tissue is generally damaged, for the occurrence of chromaffin hypoplasia with thymus hyperplasia seems to be a fact, but whether this is so true of the purely secondary types is a question, though Bartel observed that the adrenals were not infrequently found to be tuberculous in status lymphaticus. In one case in the Cincinnati Hospital, in which the symptoms of a developing Addison's disease were present, and in which there were tuberculous lesions in one adrenal, there was no evidence of any thymus tissue, even in microscopic sections. This case was a distinctly secondary one, in which there were progressive pulmonary tuberculous lesions. There were also no discoverable lesions in any of the ductless glands examined (pancreas, pituitary, pineal, thymus). The chromaffin tissue of the adrenals was hypoplastic.

More striking, however, is the appearance of enlarged thymus in Graves' disease. It was long after von Miculicz had remarked upon the presence of an enlarged thymus in exophthalmic goiter, and long after Rehn had suggested operative attentions for the thymus in such cases, that Garré and Capelle reported their striking observation that in 95 per cent of fatal cases of Graves' disease an enlarged thymus was present. Since that time the records have multiplied, and now there seems no doubt that a hyperplastic thymus is a very important factor in the disease. Hart goes so far as to state his belief that there is a pure thymogenic form of the exophthalmic goiter. Garré has said that the blood picture of Graves' disease returns to normal after removal of the thymus; that intraperitoneal injections of thymus extracts produce the blood picture which Kocher believed characteristic of Graves' disease; and that the thyroids show evidence of regressive changes after thymectomy. Gabele has said that implantation of normal thymus in thyroidectomized dogs prevents the appearance of cachexia thyropriva, and Bircher has been able to produce a typical Graves' disease by intraperitoneal implantation of fresh pathologically hyperplastic thymus. The recent observations of von Haberer, Klose and others tend to support the conception of the dual nature of Graves' disease, and the experiences of Halsted[14] tend in the same direction, although von Haberer[15] says, in agreement with Kocher, that cases of Graves' disease without thymus hyperplasia occur, but he also says that cases of hyperplastic thyroid in association with enlarged thymus glands exist without symptoms of exophthalmic goiter.

There still remains the association between thymus persistence and hyperplasia, and dwarfism, achondroplasia, and other abnormal states. Whether these are constant associations and active ones, we cannot tell—the answer must be left to the future to decide. All that seems at all definite is that the thymus seems to be a factor—to play a part—in the processes of growth and, as Romeis[16] says, the younger the animal, the surer is the growth quickening action of the gland. His experiments gave somewhat the same results as those of Gudernatsch.

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ORTHOSTATIC ALBUMINURIA*

By Arthur F. Beifeld, M.D., Chicago.

THE following is a brief clinical note concerning a case of albuminuria of the orthostatic type. The justification for presenting it rests in part upon its apparent etiology and in part upon its yielding, to a great extent at least, to surgical intervention.

Observers are not entirely in accord as to the conditions underlying orthostatic, or following Heubner,[1] philologically more correct, orthotic albuminuria. The experimental work of Jehle[2] speaks convincingly for the rôle of a lumbar lordosis in its causation, and this theory has perhaps the greatest number of adherents. Pollitzer[3] emphasizes the importance of a congenitally insufficient faucial lymphatic ring, pointing to the frequent association of angina with this form of albuminuria. Jehle, while he concedes the truth of this last fact, states that disappearance of the albuminuria, following the removal of the offending tonsils, has never been demonstrated. This assertion was made by Jehle in 1913, in a comprehensive monograph[4] on the subject.

Among other causes suggested in explanation of this phenomenon are the following: A persistently low pulse pressure in the renal glomeruli (Erlanger and Hooker[5]); Teissier's[6] view that it is a manifestation of a latent tuberculous infection—the so-called pretuberculous stage; that it is due to increased renal permeability, hence its frequent occurrence in families (Leube[7]); that it is the sequel of some infectious process; that it is an evidence, purely, of a labile vasomotor system; and, finally, that it is the result of some interference with the extrinsic renal vessels, such, for example, as would occur when a full colon caused pressure upon the renal veins when the individual assumed the erect position.

Certain predisposing factors are better known. Thus, the condition is generally considered as distinctly more common in boys than in girls, though with this Heubner[8] and Langstein[9] disagree. Furthermore, it is essentially a manifestation of adolescence. Schaps,[10] in the Berlin clinic, found 94.12 per cent of his cases between the ages of five and fifteen years. Heubner's statistics are not entirely in harmony with those of Schaps as he classifies 38 per cent of his cases between the ages of sixteen and twenty years, and 17.86 per cent between the ages of twenty-one and thirty. Finally, as shown especially by English sources, it is of very frequent occurrence.

The essentials of the case forming the basis of this report are as follows: The patient, a young medical student age twenty-one, consulted the writer after discovering in one of his laboratory courses that his urine contained albumin in large amount. The tests employed were the heat, the Heller, and the saturated sodium chlorid-acetic acid. The quantitative determination by Esbach showed three parts pro mille. This discovery was a great surprise to the young man as his general health had never been better.

As to the anamnesis, it was brought out that aside from frequent attacks of

*Read before the Clinical Society of the Michael Reese Hospital, Chicago, April 3, 1916.

tonsillitis, he had always been well. Incidentally, it is perhaps worthy of note that a brother had had a nephritis following scarlatina and that his father had succumbed to chronic Bright's disease. The mother is subject to recurrent sore throat.

Examination showed a stout and rather muscular individual 5 ft. 11½ in. in height, weighing 217 pounds. There was no lumbar lordosis. The tonsils were large and hyperemic, and rather deeply buried; and some of the tonsillar crypts contained plugs. There was no cervical adenopathy. The lungs were negative. The heart seemed somewhat enlarged, as determined by the ordinary methods, the left border being in the mammillary line. Fluoroscopically, with the narrowed screen, MR measured 4.7 cm. and ML 9.3 cm., making a transverse diameter of 14 cm., which is the upper limit of the normal for a male individual of his age, weight and height according to the tables of Dietlen.[11] There were no auscultatory anomalies.

The abdomen was without changes; and ankle edema was absent. The blood-pressure on several examinations was 120 systolic and 80 diastolic. On one occasion the systolic pressure registered 130 mm.

The urine contained albumin by the various tests mentioned above. There was present in addition a body precipitated by acetic acid in the cold. The reaction for this substance was so pronounced as to suggest that the bulk of the material producing the positive albumin tests was of this nature. This acetic acid precipitable substance, furthermore, was present even though the reaction for serum albumin was negative. Urinalysis showed only one other abnormality; viz., the almost constant presence of scattered hyalin casts.

Further study of the case demonstrated the fact that the occurrence of albumin was strictly associated with the assumption of the upright position. If the patient, having emptied his bladder on a given night, urinated into a conveniently placed receptacle the succeeding morning, the specimen contained no albumin, and also no casts. If the young man arose to urinate, or even if he lifted himself in bed to turn on the light, albumin appeared, and as a rule, casts. The amount of albumin varied, diminishing apparently as the day wore on.

In view of the plans of the patient, it was especially imperative to determine, if possible, the underlying cause of the condition. The fact that the albuminuria was postural in nature did not, of course, rule out the possibility of a renal lesion. In the opinion of some observers, Senator[12] and Langstein[13] among others, casts eliminate the diagnosis of a cyclic or orthotic albuminuria. Sahli[14] and Jehle[15] on the contrary do not regard the presence of a few hyalin casts as militating against this diagnosis; and Jehle and others have observed cases exhibiting casts which definitely proved themselves to be of the orthotic type.

According to Langstein, the presence of a body which is precipitated by acetic acid in the cold, regarded by him as nucleo-albumin, but which is probably a chondroitin-sulphuric acid, is not observed in the nephropathies, and is strongly suggestive of the orthotic nature of the condition.

As to the heart, this is to be said: An organ enlarged to the left or to the right or transversely is very frequently observed in undoubted cases of orthotic albuminuria. This is the so-called cor juvenum of Krehl and Germain Sée.[16]

In the doubtful case, an enlarged organ is to be regarded as pathological only when conjoined with other evidences of a renal lesion, particularly hypertension, a ringing aortic second sound and abnormal vessels.

The preliminary examination of the patient took place toward the end of November, 1915. In the absence of a satisfactory etiology of the condition, and particularly in view of the uncertainty as to the gravity of the affair, tonsillectomy was advised, primarily because of the evident pathology in these organs. The writer was influenced to a considerable degree also by the studies of Pollitzer relative to the frequent association of his material with recurrent anginas; though as previously mentioned, and emphasized by Jehle, no case was on record in 1913 of the subsidence of an orthotic albuminuria following the removal of the tonsils.

The tonsillectomy was performed during the holidays. Shortly after there occurred an epidemic of infections of the respiratory tract, and to this the patient fell a victim. Complicating his affection was an otitis media and mastoiditis, the latter subsiding, however, without operative interference. During this illness albumin continued to be present in the urine.

With the termination of the middle ear and mastoid infections, examination of the urine under differing postural conditions was resumed. The results of these later tests, covering a period of six weeks, can be summarized in a few words: The urine, except on one occasion, has been, and continues to be, free from albumin and other abnormal constituents. The exception occurred when the patient, in an effort to subject himself to an unusually severe test, raced up five flights of stairs. The urine collected after this exertion contained albumin by the various tests, though in this particular instance it would be difficult to say whether the albuminuria was of the type under discussion or was merely that seen so frequently after severe exertion. No casts were found.

If one is of the opinion of Jehle that a lumbar lordosis is an essential feature of all true cases of orthotic albuminuria, then the case in question cannot be regarded in this category. If, on the contrary, a lordosis is only one of several factors responsible for the condition, among which is to be emphasized infections of the faucial tonsils, then the case under discussion may be said properly to fall in this group of albuminurias, especially as the presence of such features as casts and an enlarged heart may be explained on satisfactory grounds other than the assumption of a nephropathy.

Note (February, 1917—nearly a year since the preparation of this paper).—For several months after the above noted exceptional finding of albumin, a trace of the latter was found in the urine on isolated occasions, particularly during the middle of the day. In the past seven months, however, albumin has been absent at all times and by all tests.

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DIASTOLIC PRESSURE IN THE AGED*

By L. M. Bowes, M.D., Chicago.

A LARGE majority of the diseases of old age are due to changes which take place in the blood vessels, heart, or kidneys, and which usually progress to a fatal termination as a result of cardiac failure, cerebral hemorrhage or nephritis. Therefore, it is of extreme importance that a study be made of the condition of the cardio-vascular-renal system by means of measuring the peripheral resistance. This may be accomplished by observing the diastolic pressure, which is a better index and, as it varies less than the systolic pressure, is of much greater importance.

In order to emphasize this point I wish to report 59 deaths which occurred in old people between the ages of 65 and 95, during the past four years.

Fifteen deaths were due to chronic valvular heart disease, 9 to cerebral hemorrhage, 8 to arterial disease, 6 to nephritis, 7 to cancer, 2 to accident, 5 to pneumonia, 2 to acute enteritis, 1 to diabetes, 1 to tuberculosis, and 3 to chronic bronchitis which, in all probability, were also of cardiac origin. There was a total of 35 deaths due to cardiac or arterial disease.

Nephritis was present in a number of cases but was the immediate cause of death only in six instances.

Four hundred observations were made in a series of 161 patients, all but two of whom were between 65 and 95 years of age. The point at which all sound disappeared was recorded as the diastolic pressure. Eighty-six, or 53 per cent, had diastolic pressures between 75 and 95 mm. Hg. The average diastolic pressure for those between 65 and 69 years of age was 82 mm. Hg. After 69 there was an increase up to the period between 85 and 89 when the diastolic pressure was 90 mm. Hg.

LOW DIASTOLIC PRESSURE.

There were 43 who had diastolic pressures below 75 mm. Hg. The lowest was 58 mm. and the average low was 67 mm. Hg. Any diastolic pressure below 75 mm. was considered abnormal. Eight of those with a persistent diastolic pressure below 75 mm. were normal in health. The rest were sufferers of myocarditis, rheumatism, acute enteritis, carcinoma, anemia or senile dementia, named in the order of frequency.

*For an earlier paper on Blood Pressure in the Aged, see this journal, ii. 256.

HIGH DIASTOLIC PRESSURE.

There were 32 who had persistent diastolic pressures above 95 mm. The highest was 143 and the average 110 mm. Hg. Any diastolic pressure above 95 mm. was considered abnormal.

Fifty per cent of those who had persistent high diastolic pressures presented mental symptoms such as headache, dizziness, lack of memory, confused ideas, hallucinations, cerebral atrophy, senile dementia or cerebral hemorrhage.

Nearly all had enlarged cardiac dullness, with an accentuated second aortic sound and a systolic murmur heard at the apex or at the second intercostal space.

Eight had chronic valvular heart disease, two valvular lesions associated with cerebral hemorrhage, and two valvular heart lesions with nephritis. Two others had suffered cerebral hemorrhages. Several complained of cardiac pain and tingling in the fingers and toes.

CEREBRAL HEMORRHAGE.

As a general rule a sustained hypertension of both systolic and diastolic pressures diagnosticates cerebral hemorrhage from cerebral embolism. But there is a small group of cases in which neither pressures are high. It is not an uncommon thing to see a fall of systolic and diastolic pressures following a small cerebral hemorrhage. I have seen a drop of 25 mm. in the systolic and 5 mm. Hg. in the diastolic pressures within twenty-four hours following such a hemorrhage.

There may be a temporary rise in case a checking of the hemorrhage occurs only to fall again with a recurrence of the bleeding. These cases can only be diagnosticated from cerebral embolism by following them to autopsy. The hemorrhage is quite small and usually is located in the anterior portion of the brain.

A hemorrhage that is profuse or occurs in the region of the fourth ventricle is followed by a high or increased blood pressure.

There is less danger of a blood vessel breaking when the diastolic pressure is low even if the systolic pressure is high.

The arteries were very rigid in all of the cases of cerebral hemorrhage that had low diastolic and systolic pressures.

ARTERIOSCLEROSIS.

A great deal depends upon the heart and kidneys whether or not there is an increased diastolic pressure in arteriosclerosis. When it is raised the increase is not in proportion to the systolic which causes a high pulse pressure.

There is a great deal of value to be derived from these high diastolic pressure readings because it is an index to the amount of increased peripheral resistance.

There was a slow heart action in some of the cases of marked arteriosclerosis. A pulse rate of 40 to 50 is not uncommonly associated with a diastolic pressure of 104 mm. Hg. or even higher.

On the other hand, we may not have an increased diastolic pressure. Price[1] explains this by saying that "there is less dilatation and recoil to maintain it."

Cornwall[2] states that the diastolic pressure "is usually raised when the arterial supply of the vital regions is involved, but the elevation does not seem to be so high, as a rule, as in chronic nephritis."

Whenever myocarditis develops or the limit of the cardiac reserve power has been reached we have a fall of blood pressure, both systolic and diastolic, regardless of the conditon of the arteries.

CHRONIC HEART DISEASE.

The diastolic pressure is generally low in aortic regurgitation. It is impossible, in some cases, to make an accurate reading because a sound can be heard until all pressure in the armlet is removed.

A high diastolic pressure was found in aortic stenosis. But as it was associated with marked arteriosclerosis, it was impossible to determine which influenced the diastolic pressure.

A low diastolic pressure was found in all cases of chronic myocarditis.

A difference of 10 to 20 mm. Hg. between the small and large beats was observed frequently in irregular heart action.

CHRONIC NEPHRITIS.

Chronic interstitial nephritis is the usual type of nephritis met with, and it is not common to have an uncomplicated case at this time of life.

Arteriosclerosis, chronic myocarditis and uremia are the usual complications which may modify the blood pressure. But where chronic interstitial nephritis predominates, the diastolic pressure is higher than in any other disease of old age. It is generally between 120 and 150 mm. Hg. and a higher pressure is often seen.

The heart hypertrophies in all of these cases in order to meet the increased peripheral resistance. When the limit of the cardiac reserve power has been reached, the diastolic pressure falls with, but not as rapidly as, the systolic pressure, causing a lower pulse pressure.

This explains why we have so many cases of chronic nephritis with low systolic and diastolic pressures in those past eighty.

CHRONIC TOBACCO POISONING.

In looking over the records of fifty who have been hard smokers for forty and even seventy years, I find that the blood pressure was influenced by the associated conditions rather than by the tobacco poisoning.

One woman, aged 79, who has been a chronic smoker for many years and who has marked arteriosclerosis, associated with aortic regurgitation, had a systolic pressure of 170 mm. and a diastolic pressure of 105 mm. Hg. The urine was negative.

Some old men who have been inveterate smokers nearly all of their lives had systolic pressures from 160 to 190 mm. and diastolic pressures in the eighties.

It is to be admitted that chronic tobacco poisoning causes a decreased blood pressure in many, and may in younger men, but I do not believe it to be the general rule in these aged smokers.

DIASTOLIC INDEX.

Williams and Melvin[3] state that "in doing blood pressure estimations we find it desirable to determine the diastolic index while the armlet pressure is being raised; in some (rare) cases where arm compression leads to a marked rise of blood pressure, the diastolic reading taken with a rising armlet pressure may differ from that obtained during the lowering of the armlet pressure (after determining the systolic index) by as much as 20 mm. This was especially the case where simultaneous estimations were made in the two arms. But a decided effect was sometimes observed when only one was tested. Comparison of the diastolic values with rising and falling armlet pressures may thus give important indications of the liability of the arterial pressure to vary in an abnormal way, when a large artery is closed, as in the process of systolic estimation."

This effect was observed, in a series of cases, in which only one arm was tested. There were several cases of marked arteriosclerosis, aortic and mitral regurgitation and one of aortic stenosis which did not vary in this way. Neither did any case of cerebral hemorrhage, nephritis, diabetes, cancer or chronic myocarditis, although many observations were made and some were made but a short time before death.

A variation was present from 4 mm. found in a case of chronic organic heart disease, to 16 mm., found in a case of general edema (including edema of the lungs). Other conditions showing such variations were bronchial asthma, cardiac asthma, marked flatulence, which interfered greatly with the action of the heart and lungs, and one case where the opposite arm had been amputated.

Some cases of aortic and mitral regurgitation showed this but others did not.

One woman, aged 81, with a slight paralysis as a result of a previous cerebral hemorrhage, had an aortic regurgitation.

The rising diastolic pressure was 100 and the falling diastolic pressure was 96 mm. Hg. This variation did not change in the following observations. Shortly after, decompensation took place resulting in the death of the patient.

A man, aged 83, in whom there was no difference between the rising and falling diastolic pressures, died of cerebral hemorrhage and right hemiplegia.

There has been too little work done to learn just what the diastolic index would indicate. It may prove to be of great value especially in surgery before amputation of a limb.

In conclusion I wish to state that the systolic and especially the diastolic pressures should be studied in the aged to aid in diagnoses and intelligent treatment.

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SOME RECENT STUDIES IN THE CHEMISTRY OF THE CEREBRO-SPINAL FLUID

By Charles E. Kiely, M.D., Cincinnati, Ohio.

Weston[1] has made a very careful research on the sugar content of the blood and of the cerebrospinal fluid in mental diseases, using the technic of Meyer and Bailey.[2] An ample amount of material enhances the value of the work. Twenty cases of dementia precox were examined; ten cases of maniac depressive psychosis; twenty cases of general paralysis; six cases of epileptic psychosis; and nine cases of imbecility. The research leads to the following conclusions:

The ratio of blood to spinal fluid sugar varied from 1 to 1.55 (in epileptics) to 1 to 1.72 (in paretics). In maniac depressive psychosis the average blood sugar content was 0.1215 and the spinal fluid 0.0711. A review of the table shows no constant difference between the manic and the depressed stages. In the epilepsies the figures were respectively 0.1151 and 0.0740; in imbecility, 0.1223 and 0.0723; in paresis 0.1140 and 0.0658; in dementia precox, 0.0995 and 0.0604. This last is a disease in which various endocrine glands have been under suspicion, and the sugar content of the tissues might be an important factor in the discussion were the normal not so unsettled. Weston quotes the figures of various workers compiled by Gettler and Baker.[3] Of thirteen observers the lowest minimum is 0.04 and the highest maximum 0.15,—a variation of almost 400 per cent! Pathological sugar values cannot, of course, be predicated when the normal is so uncertain. The general average of all Weston's cases was 0.1145 for the blood, and 0.0687 for the spinal fluid. The sugar content of the spinal fluid of cases of paresis treated daily with inunctions of mercury did not differ materially from that of untreated cases,—the figures were 0.0725 and 0.0718.

Levinson[4] attempted to investigate the reduction of alkalinity of pathological fluids. His technic and interpretations are open to very serious criticism, as very few of his specimens were examined within a reasonable time after withdrawal. Moreover, variations on standing were not studied in the critical period which would surely be soon after they were taken from the body. To quote verbatim (p. 258): "In order to determine the change in alkalinity going on in the fluid after it had been removed from the body different portions of the same fluid were put into the ice box—some of the tubes being plugged with cotton and some with ordinary corks. As was natural to expect, those fluids exposed to air increased their alkalinity in a short time. Of the fluids contained in corked tubes some showed a marked increase in alkalinity, others showed hardly any increase. Pathological fluids always showed an increased alkalinity on standing." Levinson then tabulates his comparisons. Some fluids were re-examined several months after their withdrawal. Two showed no variation while the remainder ranged from 9 to 40 per cent—averaging 12 per cent.

For this reason the work of Levinson seems vitiated as his tables show that of 55 fluids, 21 were not examined even the same day, and in 27 instances there is no record whatever of the time elapsing between withdrawal and examination. Concerning the relation of alkalinity to dextrose content Levinson says: "The lowest alkalinity obtained in meningitis still showed dextrose to the amount of 0.08 which is a normal amount." Weston's table compiled from six authorities varies from 0.046 to 0.080. The value of Levinson's paper is further impaired by tabulating "Insanity" as a diagnosis in a great many of his mental cases.

A third contribution to neurological chemistry is happily one of accuracy and extreme value. Nuzum and Lecount[5] determined the hydration capacity of the brain in delirium tremens as compared with other pathological conditions and with the normal. Essentially the work is a test of the theory of edema elaborated by Martin Fischer;[6] i.e., that the human tissues, being hydrophilic colloids, absorb and retain water in proportion to their degree of acidosis. In bold outline their thesis is that alcohol which is a narcotic diminishes oxygenation in the cerebral tissues, producing thereby an acidosis so that free water is absorbed and the condition we call edema exists. Nuzum and Lecount offer copious material carefully tabulated, and with ample controls. Their technic was to immerse pieces of brain tissue from cases of alcoholic delirium in distilled water for 48 hours and estimate by weighing the amount of water absorbed. In all cases the time elapsing after death is carefully tabulated. The figures for delirium tremens are compared with those from cases of other kinds, six of whom died suddenly. These last are used as instances in which acidosis could not occur, but one at least is open to criticism as the cause of death is given as asphyxia—a condition in which acidosis should develop very rapidly. The only serious objection to their conclusions—the possibility that they are only registering a postmortem acidosis—is met by control experiments on both normal and alcoholic rabbit brains tested soon after death with results confirmatory of the figures for the human. It is to be noted that in neither the human nor the rabbit tissue was there invariably a gross edema.

Specifically their results were as follows: 40 cases of general disease examined an average of 21.3 hours after death showed an average gain of water, by weight, of 64.5 per cent; 6 cases of sudden death examined an average of 24 hours after death gave the same figure—the consonance supporting these two tables as normal. In support of Fischer's claims it is to be noted that the highest figures were obtained in such cases as theoretically should exhibit acidosis, i.e., morphinism 84 per cent, cerebral hemorrhage 74.3 per cent, cerebral embolism 73.5 per cent, and uremia 71 per cent.

Coming to the principal point of the paper, we find that 20 alcoholic brains examined an average of 21.1 hours after death showed an increase of 70 per cent; brains of five rabbits chronically alcoholic, but not reproducing any picture like a tremulous delirium examined an average of 8 hours after death gave an average of 78.9 per cent; control rabbits gave only 70 per cent.

This article by Nuzum and Lecount is followed immediately by that of Hogan,[7] recording the results of treating delirium tremens as an acute acidosis of the brain. The results are a confirmation of this hypothesis.

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SPLENOMEGALIA—AUTOPSY REPORT

By John W. Shuman, M.D., Sioux City, Iowa.

THIS autopsy is reported on account of the enormous size and weight of the spleen. The clinical diagnosis was myelogenous leukemia on account of the blood picture, accompanied by marked splenic enlargement. Through the courtesy of Dr. Geo. S. Browning who had treated the case with roentgen therapy I held the postmortem.

Fig. 1.—Right lateral view of spleen, showing measurements 6x13½ inches and the notch.

Fig. 2.—Left lateral view.

Autopsy No. 231, held November 15, 1916, at 8 P.M. upon an emaciated male body, weight 125 pounds, height 5 feet and 10 inches, apparent age 30 years. Rigor mortis slight (death eight hours prior). Usual incision. Subcutaneous fat absent. Muscles pale and flabby, also bloodless. Abdomen filled with embalming fluid. Liver at costal margin.

Liver appendages negative. Small and large intestines distended. Stomach flaccid. Appendix without change.

Spleen greatly enlarged; removed with difficulty on account of many dense adhesions all over it. The upper end of this organ was at the left fifth interspace, while the lower end rested upon the floor of the pelvis. The spleen occupied the whole of the left abdomen.

The stomach opened; the whole of the mucous membrane showed pinhead-sized hemorrhagic spots. The mucous membrane of the lower ileum and that of the colon

Fig. 3.—Cross section through thickest portion.

evidenced the same. Kidneys and other intraabdominal organs evidenced no macroscopic pathological change. Heart and lungs showed no gross pathology.

SPLEEN TUMOR.

The appearance of the spleen was a bluish-red with many stellate whitish areas over its whole surface, especially the anterior. It cut with great resistance. The cut surface was unusually dry and the normal acinous picture was obliterated. Many bands of dense scar tissue were visible (Fig. 3). The measurements of the spleen were 13½ by 6 by 5¾ inches (Figs. 1 and 2). Its weight was 9 pounds.

Sections made and stained by Dr. W. G. Rowley showed myeloid transformation. The entire pulp and follicles were transformed into myelocytes (myeloish).

A QUANTITATIVE SEPARATION OF HEROIN FROM ORGANS*

By Wm. D. McNally, A.B., Chicago, Ill.

THE usual methods used for the detection of morphine fail to show the presence of heroin. A number of experiments were performed to determine the reason for certain extracts obtained in the two above cases giving the tests for morphine. To demonstrate that the normal hydrochloric acid of the stomach did not cause a deacetylization of the heroin, I added 2 c.c. of a .2 per cent hydrochloric acid, to five samples of heroin hydrochloride, and 2 c.c. of .46 per cent HCl, to three samples, each containing .1 gram of heroin hydrochloride. The eight samples with two blanks containing the same amount of acid were placed in an incubator at 98.8° F. for two hours. The samples were then titrated with N/20 NaOH. The amount necessary to neutralize the blank was deducted from the cubic centimeters of N/20 NaOH used in titrating the .1 gram sample of heroin hydrochloride, in the .2 per cent and .46 per cent HCl, and the HCl found was equivalent to the acid combined with the heroin. (Diacetylmorphine hydrochloride, $C_{17}H_{17}(O.C_2H_3O)_2ON$ .HCl+H_2O=423.68.) The acid ether extracts of the samples titrated failed to give a red color with Million's reagent, showing absence of phenolic hydroxyl group. The alkaloid recovered responded to tests for heroin, giving a melting point of 170° C.

Table I.

REAGENT	MORPHINE	MONOACETYLMORPHINE	DIACETYLMORPHINE (HEROIN)
Froehde's	Violet color, fading to bluish green	Violet, changing quickly to a blue, fading to a yellow	Crimson purple, gradually changing to blue
Marquis'	Deep purple	Purple soon fading	Brilliant violet soon fading, addition of water changes to yellow
Mandelin's	Yellow, faint violet turning to slate color.	Pale violet fading to slate color	Pale violet, changing to pink, gradually fading
Iodic Acid	Easily reduced	Easily reduced	Not reduced
Pellagri's*	Violet, emerald green on addition of dilute tincture of iodine	Same as for morphine	Responds same as for morphine
Goldsmith's Test	Negative	Positive	Odor of acetic ester
Denige's Test Modified**	Positive	Positive	Positive
Nitric Acid	Orange red changing to yellow	Same color as morphine	Dissolves alkaloid with a yellow color, changing on standing to a greenish blue, fades to yellow. The reaction is hastened by gentle heat
Ferric Chloride	Blue color	No color	No color
Prussian Blue Test†	Deep blue color	Deep blue color	No color, upon standing, a blue will appear

*From the Cook County Coroner's Laboratory, Chicago.

TABLE I (Cont'd).

REAGENT	MORPHINE	MONOACETYLMORPHINE	DIACETYLMORPHINE (HEROIN)
Urotropin and Sulphuric Acid††	Purple	Purple	Golden yellow color, changing to saffron yellow, finally blue
Chloral Hydrate and Sulphuric Acid			Yellow. Codein will give deep green
Platinic Chloride	Amorphous precipitate	Amorphous precipitate	Crystals in form of a burr, from dilutions of 1 to 100. (See Figs. 1 and 2.)
Dichromate and Sulphuric Acid	Greenish gray to dirty green		Flesh color momentarily
Wagner's	Precipitate seen under the microscope	Dilutions 1 to 100,000	Gives a precipitate 1 to 100,000, easily seen under AA objective
Melting Point	230° C.	187° C.	171° C.

*Pellagri's Reaction: Gazz. Chim. ital., 1877, vii, 297; Witthaus & Becker, 1911, iv, 989.
To the solid substance dissolved in hydrochloric acid, add a few drops of sulphuric acid, Sp. Gr. 1.84, heat to expel the hydrochloric acid, temperature about 110° C. In the presence of morphine, codein, apomorphine, and heroin, the residue is reddish violet. Cool, take up with a few drops of hydrochloric acid (1 to 5 dilution); a bright cherry red color is produced. Neutralize with sodium bicarbonate, added in powdered form. A smoky green is noticed as the point of neutralization is reached. Add a drop of very dilute alcoholic solution of iodine; a brilliant green color is obtained. The pigment is soluble in ether forming a purple solution.
Goldsmith's test, dissolve the alkaloid in alcohol in a small test tube, add a drop of sulphuric acid; gentle heat will give odor of acetic ester.
**Denige's Test: Compt. rend. Acad. d. sc., 1910, cli, 1062; Jour. Am. Med. Assn., Aug. 8, 1914, 513.
To a solution of morphine, add a few mils of H_2O_2, and a drop of strong ammonia. The solution is then stirred with a piece of copper wire, the solution becomes deep port wine tint. Delicate to .00002 gram of morphine. If the blue color formed masks the red tint, add a few drops of KCN solution at the end of the reaction.
†Prussian Blue Test: U. S. P., IX, 130.
††Urotropin Test: Allen's Commercial Organic Analysis, 1912, vi, 389.

EXPERIMENTAL WORK WITH ANIMALS.

LAB. NO.	ANIMAL	WEIGHT IN GRAMS	DOSE PER KILOGRAM
1234	Rabbit	2100	.15
1235	"	1821	.05

Rabbit No. 1235 became drowsy, after two hours appeared normal.

Rabbit No. 1234 after receiving the heroin hydrochloride, stretched out the hind extremities, then the front. The head was pulled back, short convulsive movements were noticed, which resembled those due to strychnine, but external stimuli failed to produce convulsions. The rabbit died in tetanic convulsions, from failure of respiration, five minutes after receiving the heroin hydrochloride.

The analysis of the organs of this rabbit (1234), and the organs of a dog poisoned with heroin hydrochloride are given in Table II.

TABLE II.

ORGAN	LAB. NO. 1234	LAB. NO. 1598
Brain		Trace
Heart and blood		.0120
Lungs and blood		.0072
Stomach and contents	.0369	.0296
Intestines	.0193	.0116
Liver	.0085	.0284
Kidneys		.0162
Bladder and urine	.0006	.0071

The heroin was separated from the animal tissues in the case of No. 1234, by the method given for morphine by Peterson and Haines,[1] the alkaloid giving all of the tests for morphine. The alkaloid from the stomach gave a weak test for the acetyl radical. From analysis of No. 1234, it would appear that a deacetylization had taken place in each of the organs examined. To a sample of 81 grams of fresh liver, heart, and spleen from a rabbit, was added .1 gram of heroin hydrochloride. The sample was kept in an incubator for three hours and the alkaloid separated as in sample No. 1234. The alkaloid separated gave all the tests for morphine.

Three samples of 100 grams each of human liver, containing .005 grams of heroin hydrochloride, were acidified with acetic, tartaric, and sulphuric acids respectively. The first sample was extracted with 50 per cent alcohol, the second with 25 per cent alcohol, and the third with water. The first two samples were carried through a process similar to that used in sample No. 1234, the third was extracted by a method suggested to me by Prof. John Uri Lloyd.[2] The alkaloid extracted from each sample responded to all the tests for morphine. This demonstrated that the method of extraction and separation of the alkaloid was responsible for obtaining morphine.

As a result of the above and other experiments, I present the following method for the extraction of heroin, which entails but little decomposition and loss of the alkaloid. The principle involved is the use of a weak acid, a low temperature, and the precipitation of the alkaloid by a hydrated aluminum silicate (Alcresta).

METHOD FOR SEPARATION OF HEROIN.

Extract the alkaloid from the suspected tissue by successive portions of 50 per cent alcohol acidified with a few drops of 10 per cent tartaric acid. Keep the temperature of the samples close to 50° C. Allow the combined extractions to remain in a cool place until the fat separates. The fat is separated in a separatory funnel, washed with acidified water, and the washings added to the main extractions. If the sample is allowed to stand overnight, considerable extraneous material settles to the bottom of the flask and can be removed by filtration, washing the filtrate with 50 per cent alcohol.

The alcoholic extractions are evaporated under diminished pressure, the water bath being kept well under the boiling point. The pasty residue is taken up with a little cold acidified water, and the extraneous matter filtered off. The filtrate is allowed to filter into 95 per cent alcohol; the protein substances (albumin, albumoses, peptones) and other impurities become granular on standing, and are easily filtered off. The alcohol is evaporated off under diminished pressure, until the liquid in the distilling flask amounts to about 20 mils. This residue is taken up with cold water, acidified with tartaric acid, and filtered. To the filtrate is added one gram of "alcresta." The "alcresta" is filtered off and the liquid tested for alkaloid. More of the alcresta is added in case the first portion does not remove all of the alkaloid.

The alcresta containing the alkaloid is transferred to a glass separatory funnel and extracted with successive portions of ammoniated chloroform.

The chloroform is evaporated, leaving the alkaloid free, as a residue. If any fat accompanies the alkaloid, dissolve the residue in a .5 per cent hydrochloric

acid, filter and re-extract. The alkaloid is weighed and after testing for heroin, with the color reactions (Table I), the purity can be further substantiated by volumetric methods, melting point of the double platinum salt, and the crystalline appearance of the platinum salt.

The temperature must be kept low, during extraction and evaporation, avoiding the use of sulphuric acid, otherwise the toxicological examination will show that morphine or one of its derivatives had been taken, and fail entirely to show heroin.

Table III shows the analysis of the organs of dogs poisoned with heroin hydrochloride, giving the amount, in grams, of the alkaloid recovered.

TABLE III.

ORGAN	LAB. NO. 1798	LAB. NO. 1799	LAB. NO. 2077	LAB. NO. 2078
Brain	None	Trace	None	None
Heart	Trace	.0008	.0018	.0023
Lungs			None	None
Blood	.0414	.0257	.0044	.0029
Stomach and contents	.0561	.1011	.3574	.0835
Intestines	.0236	.0481	.0613	.0187
Liver	.0112	.0590	.0042	.0078
Kidneys	.0042	.0075	.0299	.0077
Uterus	Trace			
Urine and bladder			.0411	.0508
Spleen	Trace	Trace	.0005	.0056
Total recovered	.1365	.2422	.5006	.1793

In the above cases where only a trace of alkaloid was found, the tests were characteristic for morphine. The alkaloid extracted from the liver and intestines in all the cases gave a blue with ferric chloride. The blood in No. 2078 did not give the ferric chloride or Prussian blue reactions. In tests where concentrated sulphuric acid is used, as in Husemann's, Froehde's, Marquis', Mecke's reagents, and also in Pellagri's method there is no sharp difference between heroin and morphine. The free base forms white prisms of an alkaline reaction, having a strong bitter taste. The crystals melt at 171° C., beginning to turn brown at about 165° C. In water heroin is nearly insoluble, difficultly soluble in ether and cold alcohol, very easily soluble in hot alcohol, chloroform, and benzol. The free base is precipitated from solutions by ammonia, sodium carbonate, and caustic alkali, soluble in excess of the reagent. A solution of heroin in .5 per cent HCl forms the double platinum salt[3] on the addition of platinic chloride, this is at first amorphous, then assumes the crystalline form like a burr (Figs. 1 and 2) melting point 223° C. with decomposition. The hydrochloride melts at 230° C. Gold chloride gives an amorphous salt when added to a solution of heroin hydrochloride, the salt forming in coalescent globules. According to Danckwortt and Wright,[3] the heating of a water solution of heroin splits off an acetyl group, and there remains monoacetylmorphine.

In the quantitative work all the extracts were repeatedly purified (which entailed a loss at each purification), weighed, and checked with the volumetric method of Elvove,[4] only a lower temperature was used in volatilizing the free acid. Gordin's method gave lower results than the Elvove method. The color-

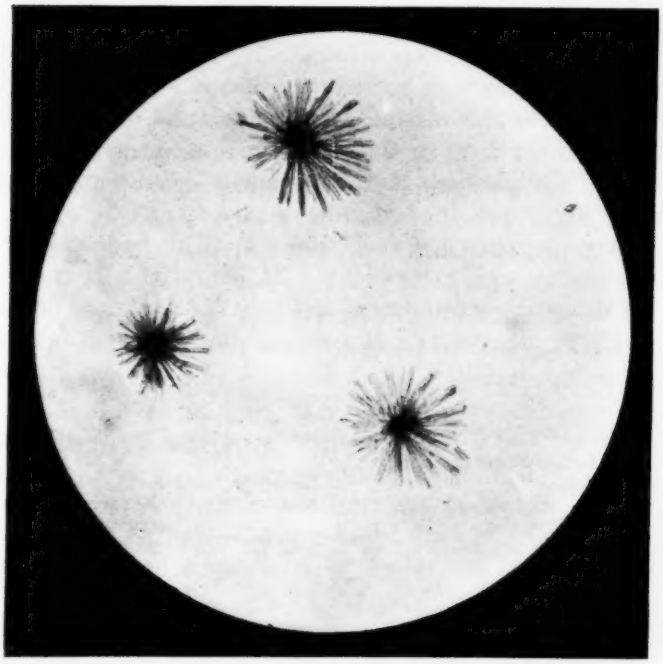

Fig. 1.—Crystals in form of burr, from Heroin Hydrochloride and Platinic Chloride. Dilutions of 1 to 100.

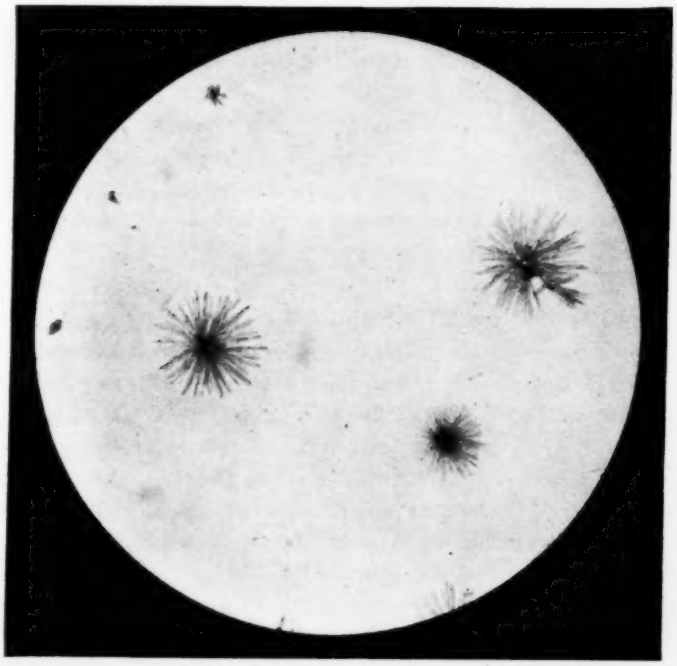

Fig. 2.—Crystals in form of burr, from Platinic Chloride and Heroin Hydrochloride. Dilutions 1 to 100. Crystals appearing in two minutes. At greater dilutions a longer time is necessary.

imetric method[5] did not give satisfactory results, as with known solutions more alkaloid was found than was actually weighed out. In most all of the extractions a brown resinous product was obtained, in only one instance was a crystalline residue obtained. Langer[6] extracted the urine of a dog with benzene, filtered the benzene, obtained in the residue after evaporation, heroin with a melting point of 168° C. In the feces of dogs given heroin, intravenously, Langer obtained morphine. The method used undoubtedly caused a deacetylization of the heroin, as the feces was dried, disintegrated, heated with alcohol for twelve hours, filtered, the filtrate evaporated, taken up with hydrochloric acid made alkaline with ammonia, and extracted for 24 hours with benzol.

I wish to express my indebtedness to Prof. W. S. Haines and Dr. John A. Wesener, for helpful suggestions and assistance in preparing this paper.

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[2]Lloyd, John Uri: Personal communication:
"Extract the alkaloid from the suspected tissues by successive extractions with dilute sulphuric acid. Add to the mixed filtrates 'alcresta' (Lloyd's Reagent a form of hydrated aluminum silicate obtained from fuller's earth). Filter the mixture, collecting the alcresta, which is extracted for the alkaloid." This method did not remove the alkaloid completely, the extracts from the tissues carrying too large an amount of extraneous material. This method was used on a sample of urine acidified with tartaric acid, the heroin being recovered unchanged.
[3]Danckwortt: Arch. f. Pharm., 1890, ccxxviii, 572.
Wright: Jour. Chem. Soc., London, 1880, xxxvii, 610; Ibid., 1874, xxvii, 1031; Ibid., 1875, xxviii, 15, 312, 689; Ibid., 1876, xxix, 652.
[4]Elvove: Hygienic Laboratory Bulletin, No. 54, July, 1909.
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PREPARATION OF PROTEIN EXTRACTS FOR DIAGNOSTIC CUTANEOUS TESTS*

By Newell S. Ferry, M.D., Detroit, Mich.

So much work is being carried on at the present time from a clinical standpoint, relative to anaphylactic skin reactions with various protein extracts, including the food, plant, pollen and microorganismal, that it has become an important and, in some respects, a serious question as to the most practical form in which to present them. This includes the best method of extracting the crude material, so that all of the available proteins are, according to Goodale[1] "in the form in which they exist when exciting symptoms," the most stable form in which to preserve them, and the most suitable and appropriate state in which to exhibit them for administration.

If the cutaneous method of diagnosticating the sensitizations to various proteins is to become of general clinical use, taking its place by the side of the tuberculin, the luetin and other tests, it is evident that the present forms in which the proteins are found should be modified.

In preparing protein extracts for use other than experimental, several things must be taken into consideration. The material, as has previously been mentioned, must be extracted in such a manner as to retain all of the proteins of the foods, plants, and pollen in an available concentrated state; the extract must be preserved from deterioration due to heat, chemical action, and bacterial contamination, and should be so mixed with a nonirritating soluble diluent that it can be used directly by the diagnostician.

There seems to be no dissenting opinion as to the value of water as an extractive, all authorities agreeing that the large majority of proteins are perfectly soluble in water, although it is recognized that such extracts are extremely liable to deterioration. To insure stability of the aqueous extracts, especially toward microorganismal contamination, which appears to be the most destructive influence, various preservatives are used and good results have been reported from all of them. Those most frequently employed are alcohol, glycerin and the phenols.

Goodale advises the use of alcohol in from 12 per cent to 15 per cent dilution, as he believes it best preserves the anaphylactogenic properties of pollen and other protein extracts. He found, however, that the pollen solutions contained one unstable albumen and a relatively stable proteose, the former being readily coagulated by exposure to air. The proteose, on the contrary, suffers but little, and will endure boiling without precipitation. These alcoholic extracts according to Goodale, "should be kept as far as possible from exposure to air, and if in the older extracts cloudiness occurs, it should not be filtered, as thereby the albumen would be removed, leaving only the proteose in solution." It is very evident, if alcohol has such an effect upon protein extracts, that some other preservative would be more acceptable especially when it is desired to use the extracts in an aqueous form.

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Several experimenters have obtained satisfactory results with carbolic acid or trikresol and it would seem that these preservatives would be more satisfactory than alcohol, as they not only will prevent bacterial contamination, but, in the small amount in which they are used, have not a deleterious action upon the proteins themselves. Carbolic acid and trikresol, or alcohol for that matter, are not ideal preservatives as the extracts in which they are used can not be applied to sensitive surfaces, such as the conjunctiva, on account of their painfully irritating action. To overcome this, H. C. Hamilton of this laboratory, has suggested an oil of high coefficiency which the writer has found best suited for this purpose, as it can be used in a dilution absolutely nonirritating to the most sensitive surfaces and still retain a germicidal action equivalent to either of the other phenols or the alcohol.

As to the methods of preparing extracts for presentation in the most practical form, several suggestions have been offered. The most feasible, up to the present time, depends upon either a direct drying of the aqueous extract or a precipitation of the proteins with alcohol or acetone, followed by a vacuum desiccation of the precipitate. Woodhouse[2] has published a method of preparing desiccated extracts which so far has given favorable results. The aqueous extract is obtained either from cooked (according to method of Goodale) or uncooked foods, and evaporated, if possible, to dryness. The residue is dissolved in sufficient water and precipitated by three or four volumes of 95 per cent alcohol. The precipitate is then centrifuged and washed several times with 95 per cent alcohol, absolute alcohol, and then ether, and finally dried over sulphuric acid. According to Woodhouse, it was necessary to deviate from this general plan by precipitating with a mixture of acetone and ether instead of 95 per cent alcohol. These methods are not entirely satisfactory as the desiccated powder is not always completely soluble in water and the more the dehydration is delayed, the more insoluble becomes the residue.

Baker and Floyd[3] have also proposed a method of desiccating the protein extracts, especially bacterial extracts, which depends upon keeping the extract at a constant temperature of 40° C. with a water bath while rapid evaporation is produced by means of a current of air from an electric fan directly over the suspension. This well-known method of evaporation is always reliable and can be used in any laboratory although the material must be well fortified against bacterial contamination. After the extract has been reduced to a powdered form, it is made into a suspension with glycerin in the proportion of 10 mgs. of the powder to 1 c.c. of glycerin. The authors have this to say concerning their mixture: "One or two drops of this preparation are used for a test. If the powder is applied to the skin directly, a constant standard can not be established or a sterile preparation can not be vouched for. The glycerinated preparation insures, with ordinary care, a sterile preparation and a constant strength, thus establishing a standard."

After trying out several of the most promising of the above methods of preparing extracts, the writer has found no one of them entirely satisfactory, the greatest fault being that they take up too much time to apply; this is quite an essential point when patients are to be tested with a large number of extracts.

The following method has been employed by the writer (the clin-

ical application of these extracts to be published later) during the past year and it has been found most satisfactory as the extracts are better preserved from deterioration and are in a form more convenient to use than any heretofore described. The protein is first extracted from the fresh or dried material with sterile water containing just enough of the previously mentioned highly coefficient oil to act as a preservative. To this aqueous extract is added enough glycerin so that when the solution is evaporated (any one of the several methods of evaporating may be used) the final amount of extract will be in a quantity sufficient to produce a standard previously decided upon. To this glycerin extract is added a sufficient amount of finely ground boric acid powder in a mortar to make a rather heavy paste. For convenience the author has had this paste put up in the ordinary small collapsible tubes, pressing out just the amount necessary for each test.

In applying the paste, the following simple and convenient procedure was suggested and has since been used to the exclusion of all others. Take upon the end of a sterile wooden toothpick just enough of the paste for a test, rubbing the paste into the scarification with the toothpick, which is then to be discarded.

The advantage of this method of preparing the various protein extracts and presenting them for administration lies in the fact that the crude material is first extracted with water, thus assuring the presence of all available proteins; the extract, which can be accurately standardized, is well preserved from deterioration, especially bacterial contamination, and is in a form suitable for instant use. The paste is perfectly miscible with, and soluble in, the body fluids or in water, is not an irritant in itself, and can be used repeatedly either with or without the collapsible tube with practically no chance of contamination.

If it is preferred to obtain the proteins in a desiccated form, according to the method of Woodhouse and others, they can be applied in the paste form by simply making a heavy suspension of the powder in the glycerin before mixing with the boric acid or by mixing the powder directly with the boric acid.

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LABORATORY METHODS

STUDIES OF COMPLEMENT: RELATION TO CERTAIN TESTS*

By Norman E. Williamson, M.D., Stockton, Calif.

IT seemed necessary to determine the value of human complement for its relation to the author's modification[1] of the Noguchi, and to the Hecht-Weinberg[2]-Gradwohl[3] Test.

The first essential to any accurate study is an exact value for the factors concerned in hemolysis. I would state the present idea of those values as follows:

A measured amount of cells. An amount of amboceptor just capable of hemolyzing these cells in the presence of a measured amount of complement obtained by pooling the serum of two or more guinea pigs. Such a conception lacks scientific accuracy, though for practical purposes it is sufficient for the performance of complement fixation tests.

The strength of guinea pig serum has in my experience varied from 0.012 c.c. to 0.03 c.c. as the Noguchi unit. By pooling, it is possible but improbable that an average value would be obtained. All of the sera may be above or below the average. I have had several successive guinea pigs yield serum decidedly above the average in strength. I spent much time in chasing this elusive unit, and have now determined a means of reaching an exact point which can always be replaced.

If one uses increasing doses of amboceptor beginning below the unit in the presence of sufficient complement for the cell suspension, hemolysis will occur steadily in proportion to the amount of amboceptor used. If, however, there is less than a unit of complement for the cell suspension, hemolysis will occur up to a certain point in orderly fashion. Above this point considerable increase in the strength of amboceptor will produce relatively trivial results in increased hemolysis. If, then, one takes several rows of tubes with amboceptor beginning below the unit, steadily increasing in strength from left to right in the same dose in each row and uses complement of different quantities in each row with an interval of complemental volume one-fourth greater than the amount in the next lower row, (provided that one of the rows shall have less complement than the unit), the result will be progressive hemolysis in this row with too little complement up to a certain point, and then relatively insignificant effect of greater quantities of amboceptor. The row above will show complete hemolysis at the point where this change is noted. This will be the exact unit of amboceptor. Exact value for complement is determined by using the intermediate values between these two rows, using 1 per cent variation, the smallest quantity producing complete hemolysis being the unit. The unit relation of factors in hemolysis might then be defined as the least amount of complement

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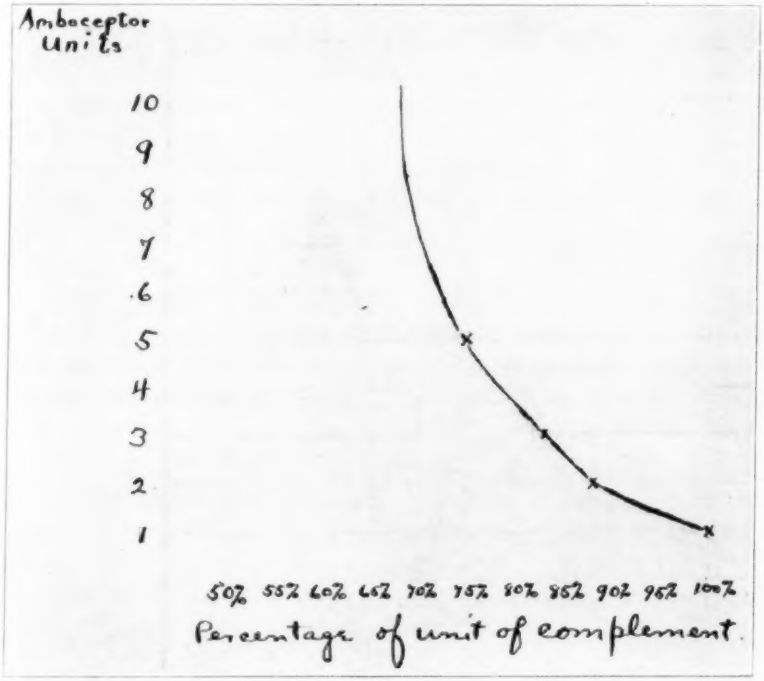
and amboceptor for a measured quantity of blood which will proceed steadily in proportion to their strength up to complete hemolysis. Taking such a unit as a basis, I have determined a curve showing the percentage of the unit of complement necessary to produce complete hemolysis in the presence of definite units of amboceptor. It will be seen that in the presence of two units of amboceptor 87.8 per cent of a unit of complement was required for hemolysis; for 3 units, 83 per cent; for 5 units, 75 per cent.

When using high unit values of amboceptor, accuracy can not be obtained though the value can be determined within reasonable limits. Bordet has explained this as deviation of complement due to union of complement to ambo-



ceptor unattached to cells. I consider it just as reasonable and more probable that it is due to the action of the foreign serum (rabbit) containing the amboceptor on the guinea pig complement. Such an action should not occur in human blood between the native antisheep amboceptor and the complement, because both are elements of the same serum. In the presence of large quantities of amboceptor, up to 30 units, no hemolysis in my experience occurred if there were less than one-fourth of a unit of complement.

Complementary values for human serum are determined as follows: First, the hemolytic power of the serum is established similarly to the method used by Gradwohl[2] in his modification, except that 0.2 c.c. of a 1 to 10 serum (0.02 c.c. of serum) and a 1 per cent suspension of sheep cells were used. The relations are the same, but this is much more economical in serum and sheep cells. Next, the units of amboceptor for the amount of cells found to be completely hemolyzed

are determined by using fractions of the serum diluted, with the same amount of cells and sufficient complement (guinea pig serum) for their hemolysis. Thus, for example, one serum in question produced complete hemolysis of 0.8 c.c. of a 1 per cent suspension of sheep cells. The same quantity of cells using sufficient complement were hemolyzed by one-fourth of the amount of serum. This serum contained 4 amboceptor units, sufficient to hemolyze 3.2 c.c. of a 1 per cent suspension of sheep cells.

Now referring to the chart, and following the line of 4 units to the curve of hemolysis and dropping a perpendicular to the base line, it will be found that 78 per cent of complement for 0.8 c.c. of sheep cells must have existed for hemolysis in the presence of 4 units. This would be the unit value for 0.6¼ c.c. practically of a 1 per cent suspension of sheep cells.

Since the amount of serum used is equivalent to the Noguchi unit when using active blood, this can be standardized by reference to a Noguchi unit of sheep cells, that is, 2 c.c. of 1 per cent suspension. Practically the complement present in the serum was 5/12 or about 0.31 of a Noguchi unit. The greatest value I have so far determined in any serum was in one which hemolyzed 0.8 c.c., and contained only two units of amboceptor. Complementary value for this serum would be 0.35 of a unit.

The measure of complement of those sera which are weak in antisheep amboceptor would be determined by adding an equal amount of serum whose values have been determined which is rich in amboceptor. The complemental value of the mixture is then determined and the difference between these and the complemental value of the known serum is the amount of complement in the serum under investigation.

It is evident as before mentioned that in the Gradwohl[3] modification of the Hecht-Weinberg[2] test hemolytic power is all that is measured by Gradwohl. It should never be forgotten that complement is the essential factor which we are measuring in all these tests. More exactly one could determine the amount of complement present in the serum used and use a just proportionate amount of antigen for an accurate test. This becomes somewhat complicated, however. The great value of the Hecht test depends on the fact that one is capable of utilizing a minimum amount of complement in proportion to the syphilitic antibody present. The effect on that complement of foreign sera is eliminated and there is only antigenic action to consider. It is conceivable in the test as they have applied it, however, that the amount of antigen in their dilutions may be less than sufficient to inhibit or absorb all of the human complement present even with a positive blood. Reactions they consider weak because they fail to appear in the high dilutions of antigen may simply lack in antigen, not syphilitic antibody. A useful, simple, qualitative test can be devised by using an amount of antigen which is more than sufficient for inhibition or absorption of the greatest amount of complement present, especially as this amount of antigen in proper dilution has an almost unmeasurable action on any complement.

I mentioned before that I found 0.35 of a unit of complement in a serum. Suppose, until wider experience has shown this to be wrong, that a possible value is assumed for a human complement of one-half the unit in 0.02 c.c. of

serum. The amount of Noguchi antigen (and, by the way, no other except the acetone insoluble fraction should be used with active blood as Noguchi has shown) which is capable of disposing in the presence of syphilitic antibody of two units of guinea pig complement plus whatever human complement may be present is 0.1 c.c. of a 1 to 10 dilution. The largest amount of antigen it would then be necessary to use would be 5/25 of 1/5 of this amount for 0.02 c.c. of serum. To overcome any physical effects of concentration it is desirable to use 0.5 c.c. of a 1 to 250 dilution. I have found, because of its sure content of antisheep amboceptor, 0.03 c.c. of serum to be a preferable amount to be used, and for this I use 0.75 c.c. of a 1 to 250 dilution. The serum is diluted 1 to 10 as before; 0.3 c.c. placed in front and back tubes, 0.75 c.c. of 1 to 250 antigen in the front tube, and 0.75 c.c. salt solution in the back tube, and incubated one-half hour. Then add 0.15 c.c. of 1 per cent suspension of sheep cells and incubate one-half hour. It is exceedingly rare to find any sera so weak in antisheep amboceptor that 0.03 will not hemolyze 0.15 c.c. of a 1 per cent suspension of sheep cells, which serve only as an index of absolute absence or presence of complement. Any quality, however small, that will show this constantly, is the right quality to use. Negatives by this test are of the highest value. Positives give little information of the amount of syphilitic antibody present, and may be erroneous. For positive bloods the simplest and best test which will show any number of units is my modification of the Noguchi test[4] and its titration sequel.[5]

Experience proves that human complement could be disregarded in this test as was stated by Noguchi,[6] though an effort was made to measure it in tube 4 with antihuman amboceptor. The fallacy of measuring human complement with cells of the same genus led to this investigation. I was anxious to determine exactly how much complement was being disregarded. I have now performed nearly a thousand tests checked by history, clinical findings, and, wherever indicated, by spinal fluid study, and find that the portions advised are satisfactory. I can not corroborate the statement of Busila[7] that no positive reaction has been obtained in the cerebral spinal fluid in a case which was negative when using active blood. I have had a case which was negative by my modification of Hecht; also by Hecht-Gradwohl and by the modification of Noguchi which gave complete inhibition using 0.2 c.c. of cerebral spinal fluid. It is necessary to study the spinal fluid in every positive or suspicious case.

Referring again to the curve, it is interesting to know its bearing on the Wassermann reaction. In the Wassermann reaction 2 units of amboceptor are used. The serum referred to above, containing 4 units for 0.8 c.c. of 1 per cent suspension of sheep cells, would have contained in the amount used in the Wassermann 3.2 units of antisheep amboceptor. There would then have been 5.2 units of antisheep amboceptor in the test. Two units require 87.8 per cent of complement for complete hemolysis. Five units require 75 per cent. The error due to excess of amboceptor in such a case is trivial. The principal objection to the Wassermann must be considered, as repeatedly emphasized, the destruction of syphilitic antibody by heating the serum. The influence of the heat on complement during the preliminary incubation in complement fixation reactions is of considerable interest. I have found that where the complement is incu-

bated with the cell suspension as in the Noguchi test for one-half hour at 37° C. from 0.4 to 0.5 of its strength is lost. This is a factor of serious moment in the titration of antigens. Not 2 units of complement, but the quantity of complement which after heating would still have a value of 1 unit, should be used in antigenic titrations. Using 2 units of amboceptor in the antigenic titration 87.8 per cent of a unit of complement would be necessary for complete hemolysis as has been shown. Probably about 15 per cent of the complement is lost as a result of the action by the antigen in the dose which proves slightly anticomplementary. The correct dose of antigen would have an anticomplementary action of from ¼ to ⅓ of this.

SUMMARY.

The standard unit of hemolysis is the least amount of complement and amboceptor which will completely hemolyze a unit suspension of blood: it can be determined by cross titration as described.

Based on this unit, a curve showing percentage of complement required for multiple units of amboceptor is presented.

By reference to this curve human complement may be measured. Hemolytic power of the serum for sheep cells is determined; amboceptor units for the same amount of cell suspension; reference to the curve gives complemental value.

Antigen just corresponding to the complement can be used in the Hecht-Weinberg-Gradwohl test instead of using hemolytic power alone as described by Gradwohl.

A more useful, simple test can be made by a modification of the Hecht, as shown, for the great value of the negatives.

For bloods containing more syphilitic antibody the author's modification of the Noguchi and its titration sequel are the best and simplest tests.

The error of the Wassermann due to excess of antisheep amboceptor is not great; inactivation is the most serious objection.

Antigens should be titrated with just sufficient complement to allow 1 active unit after heating for one-half hour at 37° C.

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EDITORIALS

The Desirability of Having Expert Entomologists in Military Camps

INSECT agencies in the spread of disease are now fully recognized. Typhus fever, yellow fever, malaria and bubonic plague are transmitted only or chiefly by the bites of insects. The specific organism of typhoid fever, dysentery, tuberculosis, and other less prevalent diseases may be transmitted by insects. In some sections of this country, notably in the Southwest, there are certain noxious insects. Apart from the transmission of disease, insects are annoyances, cause loss of sleep, and may add much to the discomfort of the soldier and greatly impair his efficiency. Moreover, insects may transmit glanders and probably other diseases among horses and certainly lead to much discomfort and loss of rest among these animals, so essential to our army. Still further, insects sometimes destroy large quantities of military stores, especially foods.

The importance of the house fly in the dissemination of typhoid fever in the military camps in 1898 was pointed out and emphasized by the special Board appointed to investigate the causes of the wide prevalence of that disease. The following is quoted from the report of the Board:

"We are satisfied that the evidence furnished in our studies, to be detailed

later, is sufficient to show beyond reasonable doubt that the most active agents in the spread of typhoid fever in many of the encampments in 1898 were flies. The reason for coming to this conclusion will be given in detail later, but may be summed up here as follows:

1. The latrines contained fecal matter specifically infected with typhoid bacilli.

2. Flies alternately visited and fed upon this infected fecal matter and the food in the mess tents. More than once it happened, when lime had been scattered over the fecal matter in the pits, that flies with their feet covered with lime were seen walking over the food.

3. Typhoid fever was much less frequent among members of messes who had their tents screened than it was among those who took no such precaution.

4. Typhoid fever gradually died out in the fall of 1898 in the encampments at Knoxville and Meade with the disappearance of the fly, and this occurred at a time of the year when in civil practice typhoid fever is generally on the increase."

The fly problem has not yet been satisfactorily disposed of. In some of the camps along the Texas border in the summer and fall of 1916 these pests were abundant, especially was this true about El Paso. Flies swarmed over and on the camps and not only annoyed the men, but according to the medical officers, a severe and widespread epidemic of amebic dysentery was undoubtedly spread by these insects. In part at least this was due to the filthy condition of the civilian population adjacent to these camps. Over these, the medical officers had no control, and local health officials were inefficient or indifferent or both. The breeding of flies in the regions about these camps rendered their exclusion impossible. This was not the only ill supplied by the surrounding civilian territory, in which red light districts and other menaces to the soldier flourished abundantly. From this we should take a lesson in establishing camps for the present war. About each camp there should be a zone, large enough to be effective, under national patrol and control. It may be that the policing of such zones should be a function of the Public Health Service. It is certain that the sanitation of such areas can not be safely left to local, civilian control.

Medical officers on the Texas border report that with flies abundant as they were last summer in some of the camps they were unable to construct fly-proof latrines. Evidently the only thing to do is to prevent the breeding of flies not only within the military lines but in the adjacent country. This is feasible, but is not likely to be done unless the sanitation of the surrounding country be placed under federal supervision. This will result in the protection of not only the military forces but of the civilian population. The army reduced the death rate in Havana below that of any city in this country and that of the canal zone below that of any State in the Union. If the sanitation of the whole country could be administered for a few years by federal authority the result would be beneficial.

The bite of the stable fly is more vicious than that of the house variety and may draw blood. It breeds in moist hay and straw and is a danger not only on account of the poison caused by its bite, which often drives horses and mules

frantic, but on account of its ability to spread disease among these animals. The charge that this fly spreads poliomyelitis is lacking in proof, but it may spread glanders and other diseases among horses. Since it does not, often at least, develop in manure, the procedures necessary to prevent its breeding are different from those efficient with the house fly.

Bedbugs sometimes render the life of the soldier at night almost unbearable and when a camp becomes badly infected the eradication of this pest is a difficult task. It has been found necessary to immerse cots bodily in a boiling dilute solution of caustic soda. So far as known this insect is not specifically responsible for the transmission of any diseases prevalent in this country, but this is a world war and there is no telling to what lands our soldiers may be called. Besides, the annoyances and irritation caused by these insects should be avoided. The cone-nose bedbug is found in Mexico. It is a blood-sucking insect, and sometimes is an inch long. It flies at night and occasionally bites man, causing much irritation and swelling and necessitating antiseptic treatment.

Fleas breed in dirt and many of the Mexican adobe houses are badly infested. Whether these fleas may spread bubonic plague or not is not definitely known.

The chigoe, not to be confused with the chigger, or bedbug, is a kind of flea. The females penetrate the skin, usually under the toe nails or on the soles of the feet, and cause marked swelling. The insect should be removed surgically and aseptically.

With the exception of the Rocky Mountain ticks, which may spread the spotted fever observed in certain localities in the West, we know of no disease in man transmitted by this insect in this country.

Centipedes carry poison glands at the base of the front pair of legs. The bite may be followed by distressing symptoms.

A poisonous spider, usually known as a tarantula, is frequent in warm countries and especially in Texas and Mexico. There is also a little black spider, with a red spot on its abdomen, whose bite is poisonous.

Some of the best camps in Texas were not free from ants, and the so-called "harvest" variety attacks man and the sting is exceedingly painful.

The soldiers in Cuba in 1898 were annoyed by a small scorpion found among the rocks. The sting is painful but not serious. This little beast seems to have a special fondness for leather and is attracted by shoes, harness, etc.

Cockroaches often come in numberless legions and may destroy much food. They seem to be wholly immune to formaldehyde, but are quickly destroyed with commercial sodium fluorid, either pure or diluted with an equal portion of some inert substance, as flour or gypsum.

Certain fly larvæ may develop in the living body, and this condition is known as myiasis, which may be intestinal or dermal. The most common insect finding its way into, and growing in, the intestines of man is the maggot of the cheese fly, usually called the cheese skipper. However, the maggots of the blow fly and the common house fly have been found in the intestines of man.

Dermal myiasis is more common and may occur in a diseased nose, or in

neglected wounds. The so-called screw-worm fly usually breeds in dead animals, but it may establish itself in wounds and has been known to cause death.

One of the most brilliant, recent discoveries is that of the French Army Surgeon, Nicolle, who demonstrated that typhus fever is transmitted from man to man by the bites of lice. Man serves as host to three species of lice. Pediculus capitis and p. pubis seem to have preempted certain portions of man's body as sources of their food supply and for the location of their homes and the breeding of their kind. The third is known as p. corporis or p. vestimenti. The former name indicates its cosmopolitan character and the latter shows that its chief abiding place is in man's clothing. This is the cause which transmits typhus fever. It was well known among the soldiers of the civil war by the appellation of "gray-back," but at that time it carried no typhus virus. This species was also abundant on the Texas border in 1916, but again was without specific infection. Soldiers should not be permitted to harbor lice of any species, with or without typhus infection. P. capitis deposits its eggs or nits on the hair some distance from the scalp and clipping the hair removes most of them. The remainder are easily destroyed by a shampoo with 2 per cent carbolic acid. One thorough inunction of its special region, with blue ointment usually destroys the pubic residents. P. vestimenti is destroyed with more difficulty and is a more dangerous guest. English and French physicians have given much attention to this parasite during the present war. Shiply has gone painstakingly into its life history, discovering its habits in feeding and breeding, etc. Others have given chief attention to means for its destruction. From what has been said it must be evident that the eradication of these pests can not depend wholly upon cleanliness of body, but the verminicides must be applied to the clothing as well. Indeed it often happens that not a louse can be found on the stripped body while the clothing may be swarming with them. Furthermore it must be evident that a verminicide suitable for application to the body may be ineffective when applied to the clothing, and since every article of clothing from head to foot, and bedding as well, may be infested, the solution of the verminicide and the method of application must vary with the fabric. In order to be effective the nits as well as the lice must be destroyed.

It is not our intention to review all the agents employed in the destruction of lice. Clothes not injured by this process, may be steamed for twenty minutes under pressure. A benzine bath, as employed in dry cleaning, is said to be effective. A soap containing 35 per cent cresol and 65 per cent naptha, and another containing 35 per cent xylol and 65 per cent naptha have been recommended by a French authority.

Yellow fever, malaria, filariasis and dengue are dissemminated by mosquitoes. The different scientific departments of the Government have issued from time to time most excellent monographs on these pests, one of which written by Ludlow is entitled "Disease-Bearing Mosquitoes of North and Central America, the West Indies and the Philippine Islands." This is most thorough up to the time of writing (1913) and we understand that a new edition is

to be issued soon. The author states that in the prevention of diseases spread by these insects two problems must be solved.

1. What disease-bearing mosquitoes are present? This divides itself into: (A) What anophelines are present; (B) Are stegomyia fasciata eulex fatigans or Mansonia uniformis and africana present? 2. How shall the species found be exterminated? It will be seen that both of these questions demand the services of an expert. Only an expert knows enough to find and identify the different species and to exterminate the species found. They should be found early and before they become numerous. Having found the breeding places, how shall the propagation of these insects be prevented? This depends upon the species and the locality. "Each land, each locality, each species presents its own problem, and until more is definitely known of the habits of the individual species, each demands a solution from the worker in his own field."

We have gone thus fully into these matters in order to show the desirability of employing the services of expert entomologists in our military camps and with our armies on the move. There is probably no country in the world better supplied with experts in this line. The Surgeon General of the Army has already rendered his country most distinguished service and made himself the greatest authority in the world in the suppression of yellow fever and malaria. There are others in the army medical corps as Craig, Darling, Russell, and Nichols who have been investigators in this field. In the Agricultural Department we have Howard and those whom he has trained, some of whom are now serving as state entomologists or as professors in universities.

The Russian army has suffered great economic loss in the destruction of its food supplies by weevils and has called upon its entomologists to prevent further loss.

The English, French, Russians, and Germans have established commissions of entomologists. We should have their expert knowledge to start with. For many years this country has recognized the importance of protecting crops from the ravages of insects. Let us have the protection and help of these specialists for our soldiers.

The following points seem clear:

1. Insect-carriers of diseases have wrought havoc in armies.
2. The early recognition of harmful insects require expert knowledge.
3. Their eradication promptly and efficiently demands the same experts.
4. The knowledge gained by a corps of skilled entomologists would make a valuable contribution to science, and be of value in the future in both civil and military life.

—*V. C. V.*

Suggestions and Recommendations Concerning the Physical Examination of Recruits for the United States Army and Navy, and for the Collection of Scientific Data and Material in Connection With the Hospital Service

THE eminent anthropologists of the Smithsonian Institution, Holmes and Hrdlicka, have submitted to the National Research Council some suggestions under the above heading. They say: "Soon a multitude of young men will be called in the organization of the new army and navy of the United States, and since good health with physical fitness are the foremost requirements of the soldier and sailor, the men called should be subjected to most careful examination. The object should be the selection not of the tallest and strongest men, but of the thousands who, on the basis of our best physical and physiological knowledge, may reasonably be expected to be fit for the tasks to be imposed upon them, or who may be made fit during the period of training. Certain standards relating to the physical development and to the health of recruits have long been established and are now employed in our recruiting offices. These differ somewhat in the different branches of service. They are all right in normal times, but they take no account of racial differences in physical characteristics and give little consideration to the possibility of improvement in the individual that should follow six or nine months of training, medical supervision, and outdoor life."

Going over the regulations for the army of the United States, these men recommend that the following "he can not speak, read and write the English language" be changed into "he does not possess a fair speaking knowledge of the English language." In other words, a man should not be excluded from the army because he can not correctly and fluently speak the English language. It is only necessary that he should be able to understand and intelligently grasp plain instructions and commands issued in this language. The present regulations say that the recruit is expected to have twenty sound teeth including four opposite incisors and four opposite molars. As the prevalent defective condition of the teeth in many cases is connected with the food and other habits of modern civilization, or to accident, rather than with disease, and as in a large majority of cases the condition of the teeth can readily be corrected by dentists, it would seem best that this stipulation be replaced by one permitting of more latitude in this direction. Possibly the following would meet the requirements: "The recruit is expected to have twenty sound teeth, including four opposite incisors and four opposite molars, but if his physical condition is otherwise satisfactory, he may be accepted even though below this dental standard, provided the defects of the teeth are such as can be readily corrected by army or navy dentists."

In regard to the stature requirements, it may be said that the present minimum in every branch of the army or navy is five feet four inches, and in the case of mountain artillery, this minimum is five feet eight inches. The minimum for the English infantry and some other branches of the service, prior to the present war, was five feet two inches, but it has since been reduced. On the Continent the minimum differs according to place. There are a number of European nationalities in which the average height of the adult male reaches but a few

tenths of an inch over the minimum of the United States. These nationalities, most of which are well represented in this country, include the Italians, Greeks, French, Swiss, many of the Slavs, Magyars, Russian and Austrian Jews, Rumanians, Lithuanians and Germans. Should the present minimum in stature for the United States army and navy be adhered to, from two-fifths to more than one-half of the men belonging to, or descendants from, the nationalities mentioned above would be excluded. In view of these facts and since short stature in a large majority of cases signifies normal variation rather than degeneration, it appears advisable that the minimum be reduced for all branches of the service to sixty inches, and that corresponding to this the minimum weight requirement be reduced from 128 to 120 pounds.

Holmes and Hrdlicka call attention to the desirability of collecting material for anthropological research during the present war. Efforts in this direction were made in the northern army of the United States during the Civil War and the results of these studies are embodied in three volumes of valuable data by Baxter and Gould, and in certain collections now in the Army Medical Museum. Our army will include not only people of many nationalities but also those of different races such as Indian, Negro, Filipino, and possibly Japanese and Chinese. By a study of both the living and the dead, information of great value may be obtained and our knowledge of anthropology may be greatly extended.

—*V. C. V.*

A New Mercurial Germicide

SCHAMBERG, Raiziss, and Kolmer, of Philadelphia, have presented to the National Research Council results which they have obtained in the preparation of a new mercurial germicide. This has been formed by the introduction of mercury into the phenol group and the trade name proposed for it is "Mercurophen." The above mentioned investigators have made a thorough study of this new compound, and have stated the results of their investigation in the following conclusions:

1. Mercurial compound No. 99 (mercurophen) exhibits against the staphylococcus aureus, in the "antiseptic test," fifty times greater activity than mercuric chloride; it destroys these bacteria on prolonged exposure in bouillon in a dilution of 1:10,000,000.

2. In a menstruum of ascitic fluid, mercurophen is two hundred times more germicidal against the staphylococcus aureus than mercuric chloride.

3. By the Rideal-Walker method, mercurophen exhibits ten thousand times greater germicidal power against the Bacillus typhosus than phenol, and over thirty times greater activity than mercuric chloride.

4. Mercurophen disinfects the hands in dilutions of from 1:10,000 to 1:40,000 in one minute, whereas mercuric chloride in a dilution of 1:5000 requires over five minutes, and in a dilution of 1:10,000 requires over fifteen minutes.

5. Mercurophen sterilizes ordinary rubber tubing in thirty minutes in a dilution of 1:100,000. Mercuric chloride accomplishes this result in a dilution

of 1:16,000. With tubing heavily infected with cocci and spores, mercurophen required a 1:500 solution and mercuric chloride fails in a 1:50 solution.

6. Mercurophen in a 1:5000 solution sterilizes feces in thirty minutes; mercuric chloride accomplishes this result in a 1:2000 solution.

7. The precipitating effect of mercuric chloride on human serum proteins is four to five times greater than that exhibited by mercurophen. This is an obvious advantage possessed by the latter substance.

8. Solutions of 1:5000 of mercurophen exhibit no evidence of tarnishing on nickel-plated instruments after twenty-four hours exposure.

9. In experiments not detailed here, mercurophen administered intravenously in rabbits has a lower toxicity than mercuric chloride. —*V. C. V.*

Digitalis Grown in America

SOME months ago the Chairman of the Committee on Medicine and Hygiene of the National Research Council took up the question of digitalis and belladonna grown in the United States. It is generally understood that we are dependent upon importation of these very valuable drugs from Europe. In the present state of medical science it would seem quite impossible in the treatment of certain diseases to get along without preparations of digitalis. It is very gratifying, therefore, to ascertain that digitalis has been grown experimentally at the Universities of Minnesota, Wisconsin, and Oregon. Wulling, Dean of the Pharmacy School of the University of Minnesota, states that he now has about one hundred quarter-pound jars of digitalis purpura of the 1916 crop. Furthermore, he adds that he would be glad to place at the disposal of the government fifteen or twenty pounds of this digitalis in coarsely powdered form. He states that this would exhaust his supply except what the hospital and dispensary of his own university would need. Concerning this year's crop he says: "We probably could arrange to increase our planting to a point to enable us to furnish at the end of the season a somewhat larger supply than we have on hand now. The powdered digitalis we have on hand would be suitable for percolating, but not for prescription work. For the latter, we could reduce the powder to a much finer state of subdivision by remilling it."

Rowntree has already published some articles on the physiological action of the digitalis grown in Minnesota. He shows that this is quite equal to, if not better therapeutically than that grown in other countries. Morris has experimented still further with the Minnesota grown digitalis.

There are many varieties of digitalis. Heretofore the variety most used is digitalis purpura, but Morris thinks that digitalis lutea is quite as efficient in its action on the circulatory organs and is much less irritating. It seems, therefore, that the Minnesota grown digitalis may in the near future wholly supplant the imported article. The necessities growing out of the present war will probably lead to many similar discoveries, and after a few years this country will be more independent than it has been in the growth of medicinal plants and the preparation of therapeutic agents from them. —*V. C. V.*